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FOLIAR APPLIED UREA NITROGEN METABOLISM IN WARM-SEASON TURFGRASS UNDER SALINITY STRESS

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ABSTRACT

The most widely used foliar nitrogen (N) source for warm-season turfgrass and agriculture is urea [$(NH_2)_2CO$], due to its low cost, high percentage of N (46% by mass), and completely soluble nature. Since urea is a soluble N source, it is commonly utilized as a foliar N source when tank mixed with pesticides in warm-season turfgrass management. The N in urea is not directly available to the plant until it is hydrolyzed into ammonia by the enzyme urease in the cytosol. Urease is a nickel (Ni^{2+}) dependant enzyme that is ubiquitous in plants. Its main biochemical function is the hydrolysis of urea; however other physiological roles have been discovered including enhancement in germination and plant defense mechanisms.

Nickel was first recognized as a required nutrient in plants in the 1970s. Critical Ni^{2+} concentrations in leaf tissue varying between 25- 100 μg kg^{-1} depending on N source and species. Nickel is a highly mobile trace element that tends to accumulate in newly formed plant parts, as well as seeds and is an important cofactor of many enzymes. Typically, excessive Ni^{2+} is a more common problem and has shown to affect physiological and biochemical processes including decreased chlorophyll, lowered photosynthetic and transpiration activities, reduced germination and impaired membrane permeability associated with enhanced extracellular peroxidase activity.

Water use and quality have become important issues in turfgrass management due to water use restrictions and mandates in arid climates. In these areas, effluent water irrigation has become commonplace, leading to potential problems with water quality including transition or heavy metal toxicity. Furthermore, seawater intrusion in coastal

areas has also led to a need for salinity tolerant turfgrasses and better knowledge of their management techniques. Although Ni^{2+} is rarely deficient in plants, the widespread use of urea as a N source in turfgrass management and the importance of urease activity requires further examination of urea N metabolism and Ni^{2+} nutrition under salinity stress.

Research also needs to examine Ni^{2+} supplementation of warm-season turfgrass supplied with combinations of $NH4^{+}$, NO_3^{-} and $[(NH_2)_2CO]$ N sources. Analysis of urea and specific amino acid concentrations in plant tissue needs to be conducted to more fully understand the uptake, assimilation, and translocation of foliar applied urea N under the influence of Ni^{2+} supplementation. The significance of Ni^{2+} supply is dependent on N source, and species. Critical Ni^{2+} concentrations in turfgrass tissues need to be determined in those scenarios. Comprehensive research of Ni^{2+} nutrition needs to be further conducted to determine the effects of supplemental Ni^{2+} levels, including Ni^{2+} toxicity symptoms, and long term ecological impact in turfgrass ecology.

Due to the lack of research examining urea N fertility, Ni^{2+} nutrition and toxicity, and salinity stress of turfgrasses, three studies were conducted. The first study examined the effect of urea fertilization method (root vs. foliar) under salinity stress of five warm-season turfgrasses. We hypothesized that urea delivery method will influence N uptake under salinity stress and the turfgrasses will perform similarly under salinity stress. Treatments included two fertility delivery methods, two salinity levels, and five warm season turfgrass genotypes. Results revealed no difference between root and foliar applications of urea N under salinity stress. There was variability in the performance of

the ultradwarf bermudagrass cultivars, with Champion exhibiting the greatest reduction in turf quality and accumulating the greatest concentration of proline in leaf tissue. Seadwarf, the most salinity tolerant genotype examined, exhibited significant increases in N concentration under foliar urea N applications and slight improvements in TQ under moderate salinity stress. In addition, foliar applications of urea N resulted in elevated Na^+ concentration in the leaf tissue of Seadwarf at the midpoint and conclusion of the study, which was the only genotype to display such a response. Findings from this study suggest that foliar applications of urea N provide an alternative to traditional granular fertilization when root zone salinity is elevated.

The second study examined urea N metabolism and the effect of Ni^{2+} supplementation on foliar uptake of urea. We hypothesized that Ni^{2+} supplementation will enhance urea N metabolism and foliar uptake by stimulating urease activity and increasing total amino acid pools in turfgrass leaf tissue. Treatments included two salinity levels, two turfgrass species and three Ni^{2+} levels. Results from this study revealed an apparent stimulation of N metabolism under foliar urea nutrition with Ni^{2+} supplementation. Although urease activity and amino acid pools were increased under Ni^{2+} supplementation, an overall decrease in N content in leaf tissue was observed over the course of the nine week study. The reduction observed in total N concentration in leaf tissue could be due to the use of a single N source (urea) causing physiological N deficiency which is a common response. Due to this finding, it is important to use multiple N sources to maintain optimal growth.

The third study further examined Ni^{2+} toxicity of two common warm-season turfgrasses under urea N fertility. We hypothesized that Ni^{2+} supplementation will stimulate urease activity and increase amino acid pools as recorded in the previous study. Secondly, as Ni^{2+} concentration in leaf tissue increases, toxicity will cause decreases in turf quality, growth, and fluctuations in micronutrient concentration. Treatments included two turfgrass species, and four Ni^{2+} levels. Results revealed a stimulation of urease activity and increases in the total amino acid pool with Ni^{2+} supplementation. However, visual toxicity symptoms occurred when Ni^{2+} concentrations increased in leaf tissue. Reductions in turf quality and growth were exhibited under 400, 800, and 1600 μM Ni^{2+} regimes. Results from this study suggest that the critical Ni^{2+} toxicity level in Diamond and TifEagle begins at a range >25 mg kg^{-1}. Ni^{2+} concentrations in leaf tissue greater than 25 mg kg^{-1} caused reductions in growth and symptoms of toxicity.

An additional fertility delivery method experiment was conducted to examine recovery of ^{15}N following root and foliar applications of urea. We hypothesized that total plant recovery of ^{15}N derived from fertilizer would be different between delivery methods and that overall recovery would be greater in foliar applied treatments. Results revealed that total plant recovery of ^{15}N labeled urea derived from fertilizer was not significantly different in either fertility regime or species tested. Although not statistically different, root applications of urea N resulted in 10% higher total ^{15}N recovery than foliar treatments at 8 hours after application. There was variability in total plant recovery across species although not statistically significant. MiniVerde displayed the lowest total ^{15}N recovery at 8 hours at 35.14%, which was much lower than Diamond and Seadwarf

at 44.66% and 47.62% respectively. Recovery of labeled urea in each plant part was significantly influenced by fertility regime, and was anticipated. Foliar applications of urea resulted in higher recovery in leaf tissue while root applications resulted in elevated ^{15}N recoveries in root tissue. In addition to recovery in specific plant tissue, root applications of urea N resulted in significantly higher ^{15}N retention in soil than foliar applications, however overall recovery of ^{15}N derived from fertilizer was higher in root treatments. The 10% overall reduction in ^{15}N recovery for foliar treatments compared to root applications could be due to a number of factors, including volatilization. The disparity, although not statistically different, in total ^{15}N recovery due to fertility regime could be biologically significant and is worth examining more closely. ^{15}N labeled urea retained in the soil (5.55%) 8 hrs after root applications has the ability to be taken up by the plant potentially increasing the overall recovery over time. Foliar treatments resulted in ^{15}N recovery in the soil of <1%. Leaching and volatilization losses were not quantified for this study, and account for the large portion of N lost when sampling took place.

Lastly, a field study was conducted to investigate the effects of N fertility levels and plant growth regulator applications on the performance of Diamond zoysiagrass as a putting green surface in the transition zone. We hypothesized that N fertility level and plant growth regulator applications would significantly influence Diamond zoysiagrass putting green performance. Results of this study revealed that Diamond zoysiagrass has the ability to become another warm-season turfgrass option for putting greens in the southern transition zone. Based on finding of this project, N fertilization of Diamond zoysiagrass in putting green scenarios should begin with 147 kg^{-1} N ha^{-1} or less over the

growing season. Additional quick release N sources should be used following cultivation events to promote growth and recovery. As total N input surpassed 147 kg^{-1} N ha^{-1} putting green performance as indicated by ball roll distance suffered. An obvious increase in thatch depth and accumulation was displayed during the two year study. Cultivation, surface management, PGR use, and fertility regimes need to be determined to optimize putting green performance and overall turfgrass health of Diamond zoysiagrass in putting green scenarios.

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CHAPTER I

INTRODUCTION

Second to water, N is most growth limiting factor in turfgrass management. Without proper N fertilization, turfgrass health, quality, and performance suffer. Comprising 3-6% of the dry weight of turfgrass, N is a vital component of chlorophyll, amino acids, proteins, nucleic acids and secondary metabolites (Beard, 1973). Turfgrass managers employ two very different techniques to apply N, granular fertilization targeting root uptake, and foliar fertilization targeting nutrient uptake through leaf tissue.

The most widely used foliar N source for warm-season turfgrass and agriculture is urea [$(NH_2)_2CO$], due to its low cost, high percentage of N (46% by weight), and completely soluble nature (McCarty, 2011). Before urea N can be useful to the plant it must be hydrolyzed by the Ni^{2+} dependent metalloenzyme urease into ammonia and carbon dioxide. Urease is ubiquitous in most vegetative plant tissue and has many functions in plant physiology including, recycling N bound in urea that accumulates during early seedling development and catabolism of arginine pools (Zonia et al., 1995). Other roles of urease are being examined, including enhancements in germination, and plant defense properties.

Ni^{2+} is a trace micronutrient that was demonstrated to be essential in plants in the 1970s. The major role of Ni^{2+} in plants is its requirement as a cofactor of urease. The influence of Ni^{2+} on urease activity and urea N metabolism has been documented in numerous species (Krogmeier et al., 1991; Gerendas et al., 1998; Moraes et al., 2009; Tan et al., 2000; Yang et al., 1996). However, literature focusing on Ni^{2+}'s influence on urea

metabolism is lacking in turfgrass where a large amount of urea is utilized every year in fertility programs.

Salinity stress is important in turfgrass management due to limitations on the availability of high quality irrigation water. Water use mandates are increasingly common, requiring turfgrass managers to use effluent water for irrigation. Coastal areas with poor water quality also can suffer seawater intrusion. The best strategy for turfgrass managers in these scenarios is to select well-adapted salinity tolerant turfgrass. The most popular warm-season putting green turfgrasses include ultradwarf bermudagrass *Cynodon dactylon (L) Pers. X C. transvaalensis* Burtt- Davy] varieties, seashore paspalum (*Paspalum vaginatum* O. Schwartz) cultivars, and most recently fine textured zoysiagrasses [*Zoysia matrella* (L.) Merr.]. Salinity stress negatively influences turfgrass health and performance in numerous ways forcing turfgrass managers to seek management techniques including fertility programs that will promote healthy turfgrass growth and minimize environmental stress.

Foliar fertilization accounts for a significant portion of total nutrients applied in turfgrass management each year. Although commonly practiced by turfgrass managers, there is still a lot to learn about the mechanisms, metabolism, and uptake of foliar applied urea N. Unlike granular applications of urea, where microorganisms in the soil break down urea into ammonium N forms prior to plant uptake, foliar applications of urea require the plant to directly hydrolyze the urea. Previous literature have examined foliar applied recovery of multiple N sources, species (Bowman and Paul, 1989; Bowman and Paul, 1990; Bowman and Paul, 1992; Henning et al., 2009; Stiegler et al., 2011) but have

not investigated Ni^{2+} nutrition in an effort to enhance urea N metabolism through augmentation of urease activity in the plant tissue.

This dissertation explores foliar and root urea N fertilization, urea N metabolism, the effects of Ni^{2+} supplementation, and Ni^{2+} toxicity in several common warm-season turfgrasses under moderate salinity stress.

CHAPTER II

LITERATURE REVIEW

Salinity Stress

The use of effluent water for irrigation is becoming more common in turfgrass management due to water use restrictions, and competition between the turfgrass industry and citizens for fresh water in arid climates. Effluent water, also known as reclaimed, gray, recycled, or wastewater are terms describing water that has gone through one cycle of domestic use (McCarty, 2011). Currently 13% of all golf courses in the United States use reclaimed water, 34 percent of them being in the Southwest (McCarty, 2011). Both limited water availability and reduced quality increase our need to examine salinity tolerant turfgrasses and management techniques (Qian et al., 2000).

Although utilization of effluent water is beneficial in terms of water conservation, it carries the risk of salinity toxicity. Turfgrasses growing in salt-affected areas suffer many salt related problems. Negative effects of utilizing effluent water as an irrigation source include diminished N metabolism resulting in reduced photosynthetic rate. Toxic element accumulation from effluent irrigation water can cause deficiencies in beneficial nutrients, most notably calcium and potassium (K^+), resulting in lower K^+/Na^+ ratios within the plant. Effluent water irrigation can lead to a more negative water potential in the soil affecting the general water balance of the plant leading to drought stress symptoms. These osmotic effects associated with salinity stress cause stomatal closure leading to reduced photosynthesis. The use of effluent water irrigation in arid climates, where it is commonly practiced can cause additional injury to turfgrass plants by causing

an accumulation of salts, including sodium chloride, leading to heightened levels of stress (Bowman et al., 2006). Salinity stress does not only affect nutrient levels within plants it has also been shown to augment N metabolism. Salinity stress can affect nutrient uptake, such as Na^+ reducing K^+ uptake and excessive Cl^- ions reducing NO_3^- uptake (Grattan and Grieve, 1999). To achieve favorable K^+/Na^+ ratios, selectivity of ions at the root zone is important. Peng et al. (2004) reported that alkali-grass (*Puccinellia* spp.) possesses a low affinity K^+ channel in the root which facilitates favorable K^+ uptake and Na^+ exclusion in salt affected sites. K^+ and sodium (Na^{2+}) selectivity is exhibited in shoot cells when of Na^+ ions are sequestered into the vacuole (Pessarakli and Kopec, 2008). If elevated levels of salt and trace micronutrients accumulate in the soil, permeability problems due to the degradation of the soil structure by Na^+ occur which can also lead to reductions in enzyme activities of microorganisms (Carrow and Duncan, 1998; Frankenberger and Bingham, 1982; Reynolds et al., 1985). Tabatabai (1977) found that many trace elements commonly found in effluent irrigation water inhibited urease activity in soil.

A change in the growth characteristics and physiology of turfgrass plants is seen under salinity stress. Moderately tolerant and salt sensitive species display reduced shoot growth in salt affected sites whereas tolerant species and halophytes often show an increase or stimulation in growth due to presumed deficiencies in salt ions. Enhanced root growth in tolerant species is frequently demonstrated under salinity stress leading to an increased root /shoot ratio that is advantageous and considered a salinity tolerance mechanism (Pessarakli and Kopec, 2008). A frequent salt tolerance mechanism of C_3 and C_4 turfgrasses is toxic ion exclusion from the shoot. Torello and Rice (1986)

demonstrated that alkali-grass (*Puccinellia* spp.) a C_3 species, restricted Na^+ ions to significantly lower levels in plant tissue than salt sensitive cultivars. Marcum and Murdoch (1994) reported that salinity tolerance of C_4 turfgrasses species depends on their ability to exclude toxic ions in shoot tissue. Ion exclusion can be facilitated through the use of salt glands or bladders which eliminate excess saline ions from shoots by active excretion (Pessarakli and Kopec, 2008). Marcum and Pessarakli (2008) found that salt glands were present in abaxial and adaxial leaf surfaces of many cultivars of bermudagrass (*Cynodon* spp.).

A difference in salt tolerance among turfgrasses has been reported in a number of studies largely based upon salinity induced growth reduction or relative turf quality (Torello and Rice, 1986). To overcome salinity stress, turfgrasses utilize several mechanisms including: compatible solute synthesis/accumulation, exclusion of saline ions at the root cortex, and excretion by salt glands (Marcum and Pessarakli, 2006). Salt exclusion has been observed in C_3 and C_4 turfgrasses with minimal osmotic adjustment in the shoot sap (Marcum and Pessarakli, 2006).

Halophytes and glycophytes adjust to increasing salinity levels by lowering tissue osmotic potentials through compatible solute accumulation (Torello and Rice, 1986). Compatible solutes are low molecular weight molecules such as glycine betaine, proline, sorbitol, mannitol, pinitol, and sucrose used by plants to adjust osmotic potential in the cytoplasm under dehydrative stresses including drought, salinity, and low temperatures (Hare et al., 1999). Compatible solutes are thought to reduce the cellular water potential below soil water values, thus maintaining turgor pressure high enough to sustain growth

under drought or salinity stress (Delauney and Verma, 1993). K^+ ions also function in a similar way by adjusting the osmotic potential within plant tissues to cope with the lowering of the water potential in salt affected soils. An N containing amino acid, proline, can be synthesized via two pathways in plants, 1) glutamate pathway the most common pathway under osmotic stress and 2) ornithine pathway, which is considered the biosynthetic pathway under supra optimal N conditions. Proline accumulation has been reported to occur after high and low temperature, transition metal toxicity, pathogen infection, anaerobiosis, nutrient deficiency, atmospheric pollution and elevated UV exposure (Verbruggen and Hermans, 2008). Proline is also thought to play a principal role as an osmoregulatory solute in plants subjected to osmotic stresses (Delauney and Verma, 1993). Increased proline contents within leaf tissue would suggest a shift in N metabolites due to the increased synthesis of the amino acid under environmental stress. Proline accumulation has been proposed to act as a way to store carbon and N (Hare and Cress, 1997).

Salt glands or bladders eliminate saline ions by active excretion and are present in many salt adapted turfgrass genera including *Cynodon* and *Zoysia* (Marcum et al., 1998; Marcum and Pessarakli, 2006). In addition to salt secretion, it is probable that salt tolerant species also compartmentalize Na^{2+} and Cl^- ions within the vacuoles while organic solutes are accumulated in the cytoplasm (Marcum and Pessarakli, 2006). Although adjustments in osmoticum are made, glycophtytic species' growth is usually inhibited due to the toxic effects of accumulated solutes. Although exclusion of Na^+ and Cl^- in the shoot is critical to maintain enzyme function and growth in turfgrasses under

salinity stress, C_4 plants utilize Na^{2+} and Cl^- for osmotic adjustment. In C_4 turfgrasses the regulation or selectivity of Na^+ and Cl^- is a better description of their tolerance mechanism under salinity stress (Pessarakli and Kopec, 2008). In C_3 and C_4 turfgrasses the maintenance of K^+/Na^+ ratio is necessary to cellular enzyme function (Marcum and Murdoch, 1994).

Uptake of N and its metabolism are restricted by root zone salinity, which increases the potential for nutrient losses due to leaching (Bowman et al., 2006). Generally, plants prefer a mixed N source (NH_4^+, NO_3^-) under salinity stress (Kant et al., 2007). Kant et al. (2007) showed that barley (*Hordeum vulgare* L.) fed a mixed N regime led to an increase in total N in control and saline environments. Supplementing ammonium for nitrate under salinity stress has been shown to alleviate deleterious effects by increasing concentrations of iron, chlorophyll and reducing sugars in tomato (*Lycopersicon esculentum* L.) (Flores et al., 2001). Uptake of nitrate in barley and nitrate and ammonium uptake in wheat (*Triticum* spp.) are affected by salinity stress (Aslam et al., 1984). N status also affected nutrient uptake in salinity-stressed tall fescue (*Festuca arundinacea* Schreb.) with 60% reductions under sufficient N regimes, whereas N deficient tall fescue absorbed nearly the entire amount of available N under salinity stress (Bowman et al., 2006).

Lewis and Chadwick (1983) found that the highest amount of ^{15}N assimilation in mixed N fed plants (most robust plants), followed by ammonium fed plants (smallest, least robust plants) and finally, the nitrate fed plants. Pease et al. (2011) found that N forms applied had negligible effects on velvet bentgrass [*Agrostis canina (*L.)] turf

quality. Bailey (1999) found that N sources affected biomass production and partitioning, under nitrate treatments, creeping bentgrass (*Agrostis palustris* subsp. *stolonifera* L.) partitioned resources into shoot and stolon formation whereas ammonium treatments preferentially partitioned resources into root production. Due to the lack of consistency in the patterns of ammonium and nitrate absorption rates with time implies that the plants had no specific preference for either N form (Bailey, 1999). Picchioni and Quiroga-Garza (1999) found that recovery of soluble ammonium nitrate and ammonium sulfate was higher than urea in 'TifGreen' bermudagrass. Total N recovered after fertilization for ammonium sulfate and ammonium nitrate averaged 78% of the applied N, whereas urea only averaged 66%. This finding is inconsistent with the suggestion that foliar urea uptake is rapid, less likely to leach. Under suboptimal growing conditions (decreasing photoperiod, reduced temperature) losses of foliar applied fertilized increased to 46%-62% of the applied N (Picchinoi and Quiroga-Garza, 1999). Contradictory results were found by Bowman and Paul (1992), where perennial ryegrass [*Lolium perenne (*L.)] fertilized with foliar urea, ammonium, and nitrate all exhibited similar uptake.

In order to be incorporated into amino acids, nucleic acids, and other compounds, the anion form of N (NO_3^-) must be reduced to NH_4^+ which is energy dependent. Nitrate fed plants showed no reduction in dry mass and only a slight reduction in fresh mass up to the 50 mM salinity levels, whereas the effect of salinity at the 20 mM level in ammonium fed plants was very marked and became increasingly so with elevated levels of salinity. Due to the different tissues in the plant where nitrate (leaf) and ammonium

(roots) are assimilated, salinity stress could have deleterious effects on ammonium metabolism and ionic effects that don't affect leaf based N assimilation. Although most salinity/nutrient related studies demonstrate that salinity reduces nutrient uptake and accumulation or affects partitioning within the plant, little evidence exists that adding nutrients at levels above those considered optimal in non-saline environments, improves crop yield (Grattan and Grieve, 1999).

Urea N Metabolism

In addition to being a popular N fertilizer source, urea is also an important N metabolite in plants (Figure 2.1) generated by arginine (Arg) degradation and ureide catabolism (Merigout et al., 2008). Before urea N can be assimilated into amino acids it must be hydrolyzed by urease into ammonia (NH_3) and carbon dioxide. Urease, a Ni^{2+} dependent cytosolic enzyme, is ubiquitous in plants and is found in soil where it can lead to considerable N loss through volatilization following urea fertilization (Torello and Wehner, 1983; Stiegler et al., 2011). Ureases are Ni^{2+} dependent metalloenzymes that catalyze the hydrolysis of urea to ammonia and carbon dioxide, enhancing the rate of the un-catalyzed reaction by a factor of 8×10^{17}. The reaction catalyzed by urease is essential to make urea N accessible to plants (Gerendas et al., 1999). The best genetic data concerning plant ureases are available for soybean (*Glycine max*) (Polacco and Holland, 1993). The embryo-specific urease is an abundant seed protein in many species including soybean and jackbean (Polacco and Holland, 1993) and Arabidopsis (Zonia et al., 1995), while the ubiquitous urease iso-enzyme is found in lower amounts in vegetative tissues of most plants (Hogan et al., 1983). The ubiquitous urease is

responsible for recycling metabolically derived urea and hydrolysis of externally generated urea, while the embryo-specific urease's role is currently unknown. (Sirko and Brodzik, 2000).

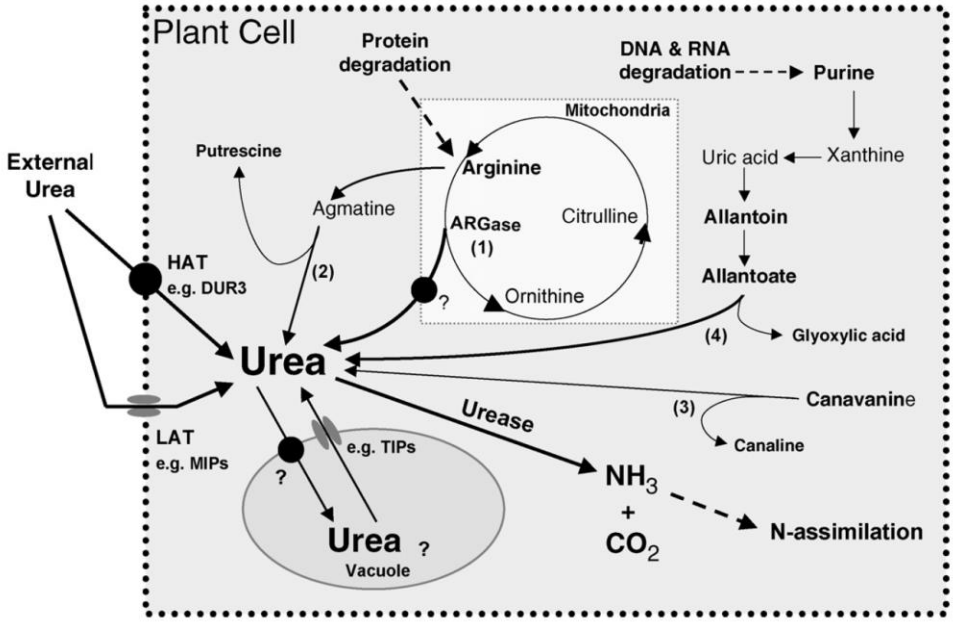


Figure 2.1 Urea generation, transport, degradation in plant cells (Wang et al., 2008). Reprinted with permission, documentation in appendix C.

Urease has many roles in plants including enhancements in germination, plant defense, but it's most well known function is its role in urea metabolism (Brown et al., 1987; Krogmeier et al., 1991; Gerendas and Sattelmacher, 1997a; Gerendas et al., 1998a; Gerendas et al., 1999). The primary role of urease is to allow the use of external or internally generated urea as an N source (Sirko and Brodzik, 2000). N present in urea is unavailable to the plant until hydrolyzed by urease and incorporated into organic compounds (Figure 2.2) by glutamine synthetase (Sirko and Brodzik, 2000). Urease has a molecular weight of 590 kDA and is the only Ni^{2+} containing metalloenzyme (Figure

2.3) yet identified in plants, and the importance of Ni^{2+} for urease activity has been demonstrated in many studies (Menegassi et al., 2008; Krogmeier et al., 1991; Sirko and Brodzik, 2000; Zonia et al., 1995).

Several studies have examined mutant, or urease antisense plants including potato (*Solanum tuberosum* L.) and soybean to further establish roles of urease within plants. A urease negative soybean mutant accumulated considerable levels of urea in all tissues and exhibited necrotic leaf tips (Krogmeier et al., 1991). Observations that urease-negative mutants also tend to germinate more slowly and at lower frequency were noted. Witte et al. (2002) demonstrated urease activity in wild type and urease-antisense transgenic potato. Urease activity in leaves of the antisense plants was approximately 30% of the controls, and urea N accumulated to higher concentrations in the antisense plants due to the lowered hydrolysis of urea. However, Witte et al. (2002) reported that urea degradation rates after the initial increase in urea concentrations are similar in both transgenic and non-transgenic plants. A correlation between urease activity and ^{15}N metabolism was found, however, there was no effect of urease activity on either N losses or ^{15}N distribution in the plants after foliar urea application (Witte et al., 2002). Lastly, urease activities per unit protein were far higher in older leaves and mother tubers (Witte et al., 2002). Conclusions from the work done by Witte et al. (2002) supports the theory that urease serves not only as a critical enzyme in the hydrolysis of foliar applied urea but for recycling N in plant tissue acting as an N source.

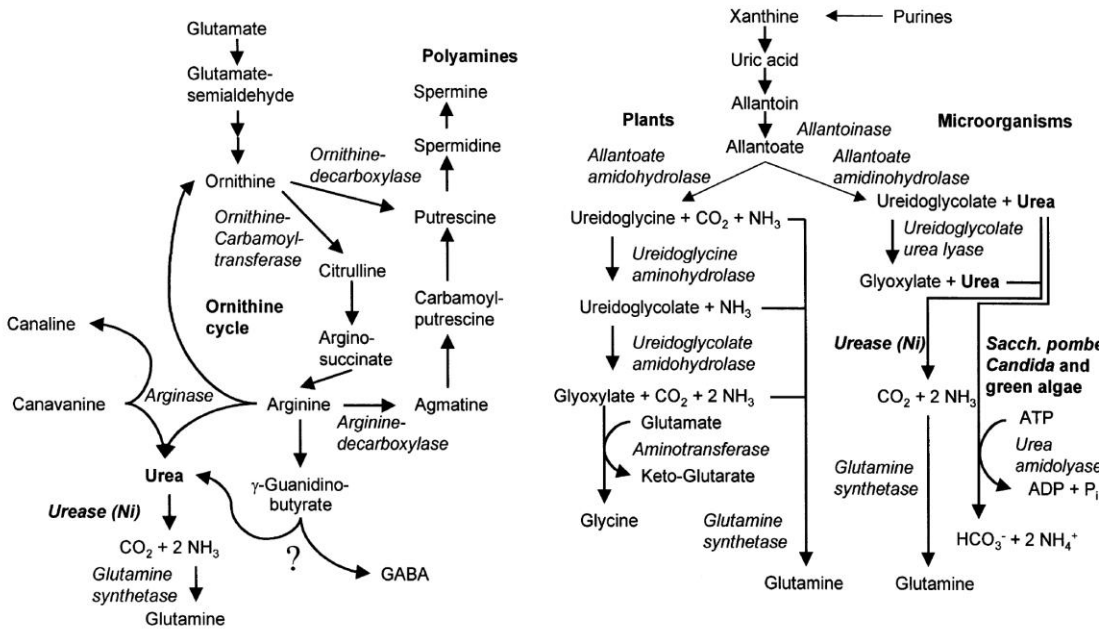


Figure 2.2 Function of urease and turnover of urea and ureides in plants and microorganisms (Gerendas et al., 1999). Reprinted with permission, documentation in appendix C.

Further examinations of Ni^{2+} nutrition and urease activity by Grenedas et al. (1998b) revealed Ni^{2+} to be irreplaceable by cobalt (Co) in maintaining functional urease activity in zucchini (*Cucurbita pepo* convar. *Giromontiina*), and soybean. Many positive effects of supplemental Ni^{2+} applications have been found through studies focusing on the importance of adequate Ni^{2+} when plants are fertilized with urea N. Yang et al. (1996) found that Ni^{2+} significantly influenced influx and transport of micronutrients in white clover (*Trifolium repens)*, cabbage (*Brassica oleracea)*, ryegrass and corn (*Zea mays* L.) under solution culture. Gerendas et al. (1998) found that rice (*Oryza sativa* L.*)* grown with urea as the N source are highly sensitive to inadequate Ni^{2+} supply, causing a reduction in dry matter production. Ni^{2+} also influences chlorophyll concentration in leaf tissue but depends on quantity and species; it can be positive for maize, oat, and potato

and detrimental for barley and tomato (Moraes et al., 2009). Tan et al. (2000) found a strong relationship with chlorophyll concentration when urea was amended with Ni^{2+}, however ammonium nitrate nutrition with and without Ni^{2+} did not demonstrate any increase in chlorophyll concentration. Additions of molybdenum (Mo) and Ni^{2+} together increased urease activity greater than each element supplied separately in rice and found that Mo and Ni^{2+} additions also increased dry matter production and when omitted reduced chlorophyll and photosynthetic rate (Moraes et al., 2009).

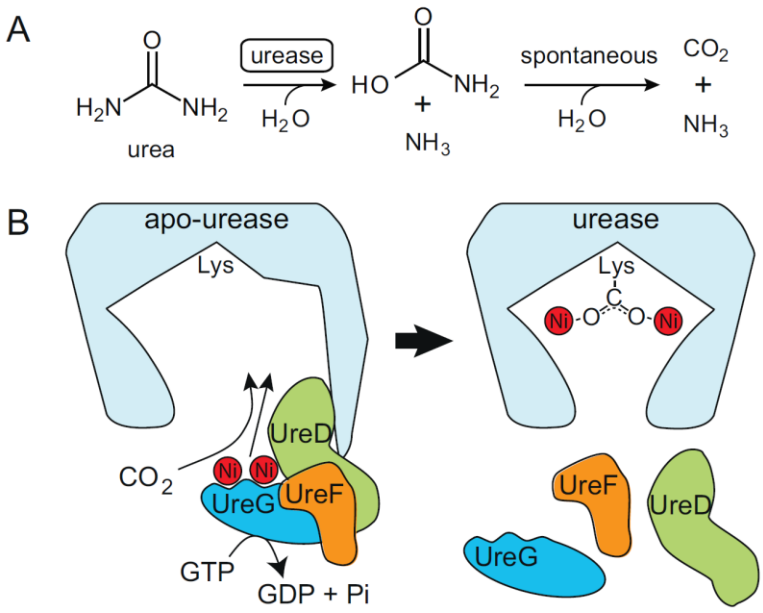


Figure 2.3 Hydrolysis of urea by urease and model of urease activation (Witte, 2011). Reprinted with permission, documentation in appendix C.

The increasing use of urea as N fertilizer calls for more detailed studies on plant urea metabolism, particularly in cases where urea is applied as a foliar fertilizer or in hydroponic systems with high purity chemicals, where Ni^{2+} supply may inadequate (FAO, 2008). Without adequate Ni^{2+} levels, urease activity in plant tissue is hardly

detectable which can lead to accumulation of urea in leaf tissue that can cause toxicity, foliar burn, and physiological N deficiency (Krogmeier et al., 1991; Gerendas et al., 1998a,b). Critical Ni^{2+} concentrations in leaf tissue vary between 25- 100 µg kg^{-1} depending on N source and species (Gerendas and Sattelmacher, 1997b; Gerendas and Sattelmacher, 1999). Tan et al. (2000) found that urea assimilation, chlorophyll content, and total leaf N in urea-fed tomato plants was significantly increased by Ni^{2+} supplementation indicating that N absorption increased. Gerendas and Sattelmacher (1999) found that spring rape (*Brassica napus* L.) grown on Ni^{2+}-deficient urea based media accumulated urea, while their amino acid content reduced, leading to an N deficient phenotype and substantial growth repression. Nickel deficiency has been shown to disrupt several metabolic pathways in pecan *[Carya illinoinensis (Wangenh.*) K.Koch] leading to abnormality in growth including stunting of leaves known as mouse ear (Wood et al., 2004a, 2004b; Bai et al., 2006). The importance of Ni^{2+} supplementation also was determined under ammonium nitrate nutrition. Spring rape and lettuce (*Lactuca sativa* L.) grown on ammonium nitrate as N source without Ni^{2+} supplementation accumulated urea in leaf tissue due to a lack of urease activity hydrolyzing metabolically generated urea (Gerendas and Sattelmacher, 1997b; Gerendas and Sattelmacher, 1999). Gerendas and Sattelmacher (1997a) demonstrated similar results in six plants grown with urea as the sole N source without Ni^{2+} supplementation, further demonstrating the importance of Ni^{2+} under urea fertility.

Leaf tip burn was also exhibited with a urease negative phenocopy induced by Ni^{2+} deprivation (Eskew et al., 1984) in which 2.5% of the dry weight of the necrotic leaf

tip was urea. Tan et al. (2000) found that supplemental Ni^{2+} applications reduced the symptom of urea toxicity in tomato seedlings. The symptoms of foliar toxicity was caused by urea rather than ammonium, the urea assimilation product, for the following reasons: as Ni^{2+} concentration increased urea N concentration decreased in leaf tissue with accompanying loss in toxicity symptoms, which looked differently from ammonium toxicity. Symptoms of urea toxicity and low N concentrations were exhibited in the urea fed tomato plants without Ni^{2+} supplement, and the symptoms were reduced in the plants with Ni^{2+} supplement at 0.01 mg L^{-1} (Tan et al., 2000). In addition to lowered foliar toxicity the lowest concentration of leaf total N was detected in the urea-fed tomato plants without Ni^{2+} supplement and urea assimilation increased, as the Ni^{2+} concentration in the solution increased from 0 to 0.1 mg L^{-1}, but no further increase at 1 mg L^{-1} (Tan et al., 2000). The changes of the concentrations of leaf urea N and NH_4 N in the urea fed plants indicate that a very rapid initial hydrolysis of urea is stimulated by the Ni^{2+} absorbed form the nutrient solution (Tan et al., 2000).

Nickel Nutrition

Nickel is an essential nutrient for plants, however the amount of Ni^{2+} required for normal growth is very low. Dixon et al. (1975) first discovered the function of Ni^{2+} for urease activation and Ni^{2+} was determined to be essential in higher plants by several authors (Eskew et al., 1983; Brown et al., 1987; Marschner, 1995). Nickel deficiency decreased the capacity for barley to develop viable seeds due to hindered embryo growth (Brown et al., 1987a,b). In addition to seed development, $Ni^{2+}$is an important component of many enzymes, where it coordinates either with S-ligands and O-ligands (urea), S-

ligands (hydrogenase) or ligands of tetrapyrrol structure (Marschner, 1995). Nickel deficient barley exhibited disrupted metabolism of amino acids, malate, and various inorganic acids. Accumulation of urea in the foliage of soybean and cowpea due to Ni^{2+} deficiency, affected amino acid metabolism, reduced urease activity, induced metabolic N deficiency, and affected amino acids, amides and urea cycle intermediates in several non woody species [rye, wheat, soybean, rape, zucchini, and sunflower (*Helianthus annuus*)] (Bai et al., 2006).

Nickel in the environment is commonly found in the form of nickelous ion Ni^{2+}. Nickel is released into the environment from anthropogenic activities including metal mining, smelting, burning of fossil fuels, vehicle emissions, and disposal of wastes, fertilizer applications and organic manures (Chen et al., 2009). The hydrated form as Ni $(H_2O)_6^{2+}$ is the most abundant form of Ni found in the soil solution (Yusuf et al., 2011). Nickel uptake in plants is carried out through the root system via passive diffusion and active uptake (Seregin and Kozhevnikova, 2006). Nickel uptake by plants depends on the concentration of Ni^{2+}, plant metabolism, the acidity of soil or solution, the presence of other metals and organic matter composition (Chen et al., 2009). Nickel uptake declines at higher pH values of the soil solution due to the formation of less soluble complexes. The uptake of heavy metals from the soil solution is strongly affected by calcium ions (Marschner, 1995). Uptake of Ni^{2+} can be inhibited by Cu^{2+} and Zn^{2+}, which indicates they may be absorbed by the same transport system (Chen et al., 2009). Nickel can also enter the plant through the leaves. Sajwan et al. (1996) found that sunflower leaves translocated 37% of the total foliar applied ^{63}Ni. Similar trends were found in oat,

soybean, tomato and egg plant [*Solanum melongena* (L.)] leaves when sprayed with a Ni^{2+} solution (Hirai et al., 1993). Nickel is transported through the plant via the transpiration stream in the xylem, typically organic acids and amino acids act as chelators to facilitate movement in the xylem (Yusuf et al., 2011). Without chelation, movement of metal cations (Ni^{2+}) in the xylem would be retarded due to the cells walls possessing a high cation exchange capacity (Yusuf et al., 2011). Nickel is a highly mobile trace element that tends to accumulate in newly formed plant parts, as well as seeds (Yusuf et al., 2011).

Nickel Toxicity

Plants rely on a range of transition (heavy) metals as essential micronutrients for normal growth and development (Yusuf et al., 2011). These elements are essential for most redox reactions which in turn are fundamental to higher functions within the plant. Although necessary, these transition metals, when accumulated above a permissible limit begin to interfere with cellular functions and inhibit normal plant metabolism causing cellular injuries and in sometimes cases death (Yusuf et al., 2011). At least three events that play a pivotal role in generating heavy metal toxicity in plants includes (1) displacement of essential components in the biomolecules by the metal, (2) blocking of essential biological functional group of the molecules and (3) modification of enzyme/proteins, plasma membrane and or membrane transporters structure/function (Ochiai, 1977). Pathways of Ni^{2+} toxicity in plants: (i) interference with other essential metal ions and (ii) induction of oxidative stress.

Nickel disrupts photosynthesis in isolated chloroplasts and in whole plants by damaging the photosynthetic apparatus at almost every level of its organization including destroying mesophyll and epidermal tissues and decreasing total chlorophyll content (Chen et al., 2009). Nickel also damages the thylakoid membrane and chloroplast grana structure (Chen et al., 2002). Nickel disrupts the light harvesting complex II and the amounts of xanthopylls and caratenoids that can lead to oxidative stress due to the increased production of free radicals (Chen at al., 2002). Enzymes such as superoxide dismutase (SOD) and catalase (CAT) are metalloenzymes containing Fe, Cu^{2+}, Zn^{2+}, or Mn^{2+} in their prosthetic groups (Chen et al., 2002). Excessive Ni^{2+} has been shown to decrease the concentrations of these metals in plant tissue, which can lead to reductions in the biosynthesis and activities of these enzymes. Increasing levels of oxidative stress have been documented by excessive Ni^{2+} in plant tissue. Significant increases in hydroxyl radicals, superoxide anions, nitric oxide and hydrogen peroxide have been exhibited in plants (Chen et al., 2002). This increase in oxidative stress is not direct, but by decreasing the number of antioxidant enzymes, reducing the plants capability to scavenge radical oxygen species (ROS), leading to ROS accumulation and finally oxidative stress (Chen et al., 2002).

Typically, excessive Ni^{2+} is a more common problem and has shown to affect physiological and biochemical processes including decreased chlorophyll, lowered photosynthetic and transpiration activities, reduced germination and impaired membrane permeability associated with enhanced extracellular peroxidase activity (Ashraf et al., 2011; Yang et al., 1996). Nickel toxicity symptoms include: mottling and necrosis of

leaves and stunted growth of shoots and roots are due to nutrient imbalances and deficiencies. Serpentine soils, commonly derived from ultramafic, or Mg^{2+} and Fe rich rocks, such as peridotites, dunites and serpentinites contain high levels of Ni^{2+}, Fe, Mg^{2+}, Co and Cr, but very low in calcium (Reeves et al., 1999). Plants found on these soils tolerant toxic levels of Ni^{2+} through incorporation with organic acids include many species exhibiting hyperaccumulation behavior where 10-30 mg^{-1} g^{-1} Ni^{2+} concentrations are not uncommon (Marschner, 1995). In most plants Ni^{2+} content in vegetative organs is between 1-10 μg g^{-1} dry weight (Marschner, 1995).

Warm-Season Turfgrasses

Golf course managers in the United States have historically established creeping bentgrass (*Agrostis stolonifera* L.) for putting green surfaces in the transition zone. However, managing quality creeping bentgrass putting greens in hot and humid climates is challenging and costly, forcing turfgrass managers to look for alternatives (McCarty et al., 2011). Therefore, other species have been introduced, evaluated, and installed to replace cool-season turfgrass putting green surfaces including ultradwarf varieties of bermudagrass, seashore paspalum, and fine leaf zoysiagrass varieties.

The ultradwarf bermudagrass varieties are vegetatively propagated turfgrasses that provide finer leaf texture, greater density, and superior playing conditions than 'Tifdwarf' and other older dwarf varieties of bermudagrass. In addition, the hybrid bermudagrasses are able to withstand high temperatures and drought events better than creeping bentgrass (McCarty et al., 2011). The three most popular varieties of ultradwarf bermudagrass in the transition zone and further south are 'TifEagle', 'MiniVerde' and

'Champion'. TifEagle was derived from a cobalt radiation induced mutant, whereas Champion and MiniVerde were developed from field selections (Hanna and Elsner, 1999).

Although the ultradwarf varieties offer improved turf quality and playability than older cultivars, they do present new management issues including thatch development (Hollingsworth et al., 2005). Many studies have focused on putting green management of hybrid bermudagrass putting greens including use of plant growth regulars (PGR) and N rates (McCarty et al., 2011; McCullough et al., 2004; 2005; 2006; 2007), cultural management, establishment, and mowing height (Guertal and Evans, 2006; Hollingsworth et al., 2005) daily light requirements (Bunnell et al., 2005) and salinity tolerance (Marcum and Pessarakli, 2006).

McCarty et al. (2011) determined that low doses of foliar applied trinexapac-ethyl (TE) resulted in increased root mass of TifEagle agreeing with findings by McCullough et al. (2004) under greenhouse conditions. In addition to increased root mass under PGR programs, McCullough et al. (2006) found that applications of TE enhanced color and nutrient retention in TifEagle rhizomes. During establishment, Guertal and Evans (2006) found that TifEagle exhibited the greatest ground cover and shoot density at N rates of 3.4- 4.3 g N m^{-2} wk^{-1} and mowing heights greater than 3.2 mm. Findings by Hollingsworth et al. (2005) found that the ultradwarf varieties may not require frequent, deep vertical mowing for thatch control that previous dwarf bermudagrasses needed. Bunnell et al. (2005) found that TifEagle bermudagrass requires a daily light integral of > 32.6 mol m^{-2} d^{-1} to maintain acceptable turfgrass quality and performance. In addition, it

appears that afternoon shade can be extremely damaging to TifEagle growth (Bunnell et al., 2005).

'Diamond' zoysiagrass [*Zoysia matrella* (L.) Merr.] is a highly rhizomatous and stoloniferous vegetatively propagated selection from Texas A&M University that has gained popularity on golf courses in the southern transition zone due to fine texture and tolerance to shade, salt, and wear (Qian and Engelke, 1999; Qian et al., 2000; Stiglbauer et al., 2009). Currently there are six golf courses in the Carolinas with Diamond zoysiagrass putting greens (Personal communication, New Life Turf). Zoysiagrasses are gaining popularity on golf courses due to their excellent wear tolerance, slow growth rate, improved winter hardiness, and unique green color during the summer (McCarty, 2011). Sladek et. al (2009) demonstrated that Diamond zoysiagrass exhibited excellent turf quality under 50% shade, outperforming other zoysiagrass varieties.

A potential shortcoming of using Diamond zoysiagrass as putting greens is its slow establishment rate. Patton et al. (2007) showed that Diamond zoysiagrass had the slowest establishment rate of *Z. matrella* varieties due to the difference in partitioning of dry matter to stems instead of leaves. Although slow to establish, Stiglbauer et al. (2009) demonstrated that Diamond zoysiagrass can be established from sprigs in one growing season and meet putting green expectations. Establishment time can be reduced sprigging rates greater than 91 m^{-3} ha^{-1} and total N input between 20-35 g M^{-2} $year^{-1}$ (Stiglbauer et al., 2009).

Seashore paspalum is a perennial warm-season turfgrass, and is native to tropical and sub-tropical areas (Turgeon, 2011). Of the C_4 turfgrasses used on golf courses,

seashore paspalum is the most salt tolerant, and has potential to proliferate in the turfgrass industry. Seashore paspalum is considered salinity tolerant (0-20 dsM^{-1}), whereas the hybrid bermudagrasses are considered moderately tolerant at a range of (0-12 dsM^{-1}) (Carrow et al., 2001).

Maintenance of aesthetically pleasing and playable putting surfaces requires extensive management and cultivation (Salaiz et al., 1995; Hartwiger et al., 2001; Hollingsworth et al., 2005; McCarty et al., 2007; McCullough et al., 2007). A common problem in putting green management is an accumulation of thatch. Thatch is a layer of living and dead plant tissue that develops between turfgrass shoot tissue and the soil surface (Turgeon, 2011). Comprising of stem, crown, and stolon tissue, thatch typically contains high lignin contents and is resistant to decay (Beard, 1973). Thatch develops when the accumulation rate of dead organic matter from the actively growing turf exceeds the rate of decomposition (Beard, 1973). Problems associated with excessive thatch include: increased disease and insect problems, localized dry spots, scalping, and decreased heat, cold, and drought hardiness. Thatch management can be achieved through several cultivation methods including, aeration and removal of cores, vertical mowing (verticutting), and topdressing. Other chemical management techniques have been evaluated to control thatch accumulation however, McCarty et al. (2007) found that chemical management of thatch was not as effective as traditional cultivation and topdressing combined.

Ball roll distance (BRD) is an important parameter measuring putting green performance and can be influenced by: grass selection, mowing practices, fertilization,

aeration, topdressing, brushing/grooming, PGR application, water management, and surface rolling (McCarty, 2011). Koeritz and Stier (2009) found that BRD of creeping bentgrass and velvet bentgrass was consistently reduced when height of cut (HOC) increased. Application of N increases turf shoot growth, and results in wider and more succulent leaf blades with slower BRD. Rolling golf putting greens is a maintenance practice intended to provide a smooth and uniform putting surface with less resistance to BRD (Hartwiger et al., 2001). Hartwiger et al. (2001) showed that benefits of rolling were seen 48 hours after rolling treatments were applied. In addition to providing increases in BRD, rolling can also be substituted for mowing. Throssel (1981) reported a 20-25 cm increase in BRD after mowing whereas rolling alone increased ball roll 13-20 cm. Cultural practices, including excessively low mowing heights, low N fertilization, and reduced irrigation can increase the BRD on putting surfaces, but often conflict with sound agronomic principles (Hartwiger, 2001). Prolonged management of BRD by lowered HOC alone will result in undesirable putting green performance and increased turfgrass stresses.

Due to the problem with relying on HOC alone to achieve increased BRD, applications of plant growth regulators (PGR) are commonly used in putting green management. Trinexapac-ethyl (TE) is a commonly used PGR in turfgrass management. TE inhibits the plant's ability to elongate by blocking gibberellic acid biosynthesis (Baldwin, 2008). Typically, TE is utilized in turfgrass management to reduce mowing frequency, and increase playability on putting surfaces. TE effectively inhibits GA_{20} to GA_1 production late in the mevalonic acid pathway suppressing shoot vertical growth.

Research has shown that PGR use in turfgrass management enhances turfgrass quality, playability and cultural practices: increases ball roll, reduces mowing frequency, decreases weed pressure, enhances disease resistance, reduces turfgrass encroachment and enhances turfgrass establishment (Baldwin, 2008). The benefit of increased BRD on creeping bentgrass and ultradwarf bermudagrass putting surfaces by applications of TE has been examined (McCullough et al., 2007; McCarty et al., 2011). The benefits of TE applications on Diamond zoysiagrass are also documented. Qian and Engelke (1999) hypothesized that improved plant carbohydrate reserves, improved root system, increased canopy photosynthesis and enhanced turf quality can be achieved through applications of TE under shade stress. Applications of TE on Diamond zoysiagrass reduced vertical growth, leading to lowered clipping yields, and higher levels of nonstructural carbohydrates leading to a potential increase in shade tolerance (Qian and Engelke, 1999). TE applications increased root length density, provided excellent TQ, reduced vertical growth and did not reduce lateral re-growth and increased BRD compared to control treatments (McCarty et al., 2011).

Foliar Fertilization

Fertilization is traditionally conducted by two delivery techniques: granular application, targeting root absorption and liquid fertilization relying on leaf uptake of nutrients. The development and use of foliar fertilization by the ChemLawn Corporation in the 1960s revolutionized the home lawn care industry (McIver, 1990). Foliar fertilization provides several advantages to the turfgrass manager including a quick color response, easy modification of nutrient content, and the ability to mix with pesticides and

micronutrients. (Totten et al., 2008). During a two year study, similar root masses were recorded in creeping bentgrass and hybrid bermudagrass putting greens under 100% foliar fertility and 100% granular fertility programs respectively (Liu et al., 2008). In addition, Liu et al. (2008) found that foliar N fertilization didn't cause reductions in root growth.

The main downside to foliar fertilization is the inability to apply large amounts of macronutrients (N, P, and K^+) without the possibility of foliar burn and reduction in turfgrass quality (Totten et al., 2008). To overcome this problem, turfgrass managers commonly "spoon feed" low concentrations of N in short intervals by foliar sprays to maintain uniform growth and reduce the risk of foliar injury. Uptake of foliar applied nutrients depends greatly on several factors including but not limited to; chemical composition and concentration of the nutrient applied, influence of wetting agents, age of leaf tissue, and environmental conditions (Neumann, 1988). The most widely used foliar N source for warm-season turfgrass and agriculture is urea [$(NH_2)_2CO$], due to its low cost, high percentage of N (46% by mass), and completely soluble nature. Some studies have suggested that urea is absorbed more rapidly by leaves than other N sources presumably because non-polar uncharged molecular structures, such as urea, diffuse through the cuticle more readily (Wittwer et al., 1959).

Nuemann (1988) reported that an increase in the concentration of foliar applied K^+ induces a slight decrease in uptake expressed as a percentage of applied amount, but a large increase in uptake in terms of weight of K^+. Wetting agents and leaf age also influence the uptake of foliar applied nutrients due to the reduction in contact angle of the

solution applied and by the accumulation of total wax and lowered metabolism of older leaves. Lastly, environmental factors also influence the ability of ion transport across the cuticle and into leaf mesophyll. Schreiber (2001) reported that an increase in humidity from 2 to 100% resulted in an increase of cuticular water flow by a factor between 2 and 3 which would presumably influence the rate of which foliar applied taken up by the plant.

There are two major hypotheses on the pathway of uptake of foliar absorbed chemicals; 1) uptake through stomata or 2) across the plant cuticle, facilitated by hydrophilic pores in the cuticle and/or through cracks in the cuticular wax. Eichert and Goldbach (2008) reported through the use of confocal microscopy and fluorescent tracers that penetration of solutes via stomata can occur by diffusion along the pore surfaces without infiltration. Numerous studies support the role of stomata in the foliar uptake of solutes (Fernandez and Eichert, 2009). According to the solubility and mobility model of cuticular penetration, diffusion of charged substances like organic ionic compounds and inorganic ions across the lipophilic transport barrier of cuticles should not be possible, however numerous studies have quantified uptake of such solutes over the past 50 years (Riederer and Muller, 2006). Recent research examining the uptake of charged organic molecules has convincingly shown that polar and charged compounds in fact can penetrate isolated astomatous cuticles as well as intact stomatous leaf surfaces (Riederer and Muller, 2006). Numerous studies have concluded that ion uptake and movement through this means of absorption relies on polar pores traversing the cutin polymer and the waxy transport barrier.

Tan et al. (1999) showed that ^{15}N assimilation was quickest in urea-fed plants, and after 48 hours, assimilation in the whole plant was up to 76.9 % in urea fed plants but only 33.7% and 43% in the nitrate and ammonium applied plants respectively. Urea is an appropriate foliar N source due to its low ability to injure foliage, its rapid absorption and translocation, fast assimilation and the wide and suitable range of solution pH (Tan et al., 1999). Stiegler et al. (2011) found that foliar applied urea provided an efficient mode of N delivery with minimal losses due to volatilization. Due to these properties foliar fertilization of urea N accounts for a large portion of total fertility programs on golf courses and sports fields each year. Through the use of foliar fertilization, play on the golf course putting greens can commence shortly after application whereas granular fertilizers would hinder aesthetics and playability.

Slow release or granular fertilizer applications on the putting surface requires watering in, incorporation after cultivation (aeration, slicing) or long term persistence on the putting surface until absorption/breakdown, which would reduce putting green quality (Schlossberg and Schmidt, 2007). In addition to its obvious playability factor on golf courses, foliar fertilization provides many benefits, including reduced leaching, quick absorption, and more uniform growth. Foliar fertility also allows managers tank mix with other pesticides, correct nutrient deficiencies quickly and achieve increased uniformity of application. Totten et al. (2008) found that a fertility program utilizing both foliar and granular fertilization was superior compared to relying on one method exclusively on creeping bentgrass putting greens. Due to these facts, a combination of granular and foliar fertilizations has been adopted by many turfgrass managers depending

on the season and turf growth conditions (Liu et al., 2008). Lastly, absorption of foliar applications of nutrients are not hindered by soil problems that can lead to reduced levels of uptake (biological implications, enzyme activities, leaching, water content, binding, etc.).

However, diverse responses have been recorded in literature examining the recovery of ^{15}N among three N sources (NO_3^-, NH_4^+, $[(NH_2)_2CO]$. Picchioni and Quiroga-Garza (1999) found that recovery of soluble ammonium nitrate and ammonium sulfate was higher than urea in 'TifGreen' bermudagrass. Total N recovered after fertilization for ammonium sulfate and ammonium nitrate averaged 78% of the applied N, whereas urea only averaged 66%. This finding is inconsistent with the suggestion that foliar urea uptake is rapid, and less likely to leach. In addition, under suboptimal growing conditions (decreasing photoperiod, reduced temperature) losses of foliar applied fertilized increased to 46%-62% of the applied N (Picchinoi and Quiroga-Garza, 1999). Different results were found by Bowman and Paul (1992), where perennial ryegrass fertilized with foliar urea, ammonium, and nitrate all exhibited similar uptake.

CHAPTER III

FOLIAR AND ROOT UREA-N FERTILITY OF FIVE WARM-SEASON TURFGRASSES UNDER SALINITY STRESS

Introduction

Commonly, fertilization of turfgrasses is achieved in two very different ways, granular and foliar applications. Granular fertilization is a common practice in home lawn and lower maintenance turfgrass areas. Granular fertilization targets root absorption and allows turfgrass managers to apply greater amounts of each nutrient than foliar fertilization at one given event and typically have greater longevities. Foliar fertilization is typically practiced on intensively managed turfgrass areas including sports fields and golf courses (McCarty, 2011). Foliar fertilization utilizes soluble mineral nutrient sources applied to the leaf tissue of target plants. This method of fertilization gives turfgrass managers the ability to quickly address nutrient deficiencies with precision and the ability to tank mix fertilizers and pesticides together.

Water quality and use has become an important issue in turfgrass management. Turfgrass managers in coastal areas have to deal with water quality issues. In addition, in arid environments, where water use mandates and effluent water are being utilized to conserve water use, turfgrass managers need management techniques to deal with salinity stress and poor water quality for irrigation.

Proper turfgrass selection is of paramount importance when managing golf courses and sports fields under environmental stresses. Five warm-season turfgrasses examined in this study: three ultradwarf bermudagrass cultivars TifEagle, Champion, and

MiniVerde, Diamond zoysiagrass, and Seadwarf seashore paspalum. These cultivars were chosen based upon their salinity tolerance, turf quality, fine texture, and overall performance in many turfgrass scenarios.

Due to the popularity of urea as an N source in turfgrass management, this study was conducted to determine if the delivery method significantly influenced turfgrass performance and physiology while under salinity stress. The objectives of this project were 1) examine the nutrient status of five popular warm-season putting green turfgrass species in response to salinity stress, 2) identify physiological changes in each species in response to salinity stress and 3) determine the "best" delivery method of urea N (foliar, or root).

Materials and Methods

Plant Materials

Experiments were conducted at the Clemson University Greenhouse Complex, Clemson SC, from July-October 2009 (Run 1) and July-October 2010 (Run 2). Twenty 15.24 cm (2009) and 20.32 cm (2010) plugs of TifEagle, Champion, MiniVerde ultradwarf bermudagrasses, Seadwarf seashore paspalum, and Diamond zoysiagrass were harvested and washed free of soil from the research plots at Clemson and transplanted into 25cm x 45cm polyvinyl chloride lysimeters containing 85%:15% (v:v) sand:peat mixture to USGA specifications and 10.16 cm of gravel for drainage (USGA Green Section Staff, 1993). Soil chemical properties are displayed in Table 3.1. The turfgrass samples were established and acclimatized in the greenhouse for two months before treatments commenced.

Plant Culture and Treatment Procedures

During the grow-in, fertilization was applied with 10N-1.3P-4.2K and 5N-0P-5.8K liquid fertilizers (Progressive Turf Fertilizer LLC., Ball Ground, GA) @ 14.65 kg^{-1} N ha with a CO_2 pressurized backpack sprayer with a TeeJet 8002 VS nozzle calibrated to deliver 560 L^{-1} ha twice and once at 9.75 kg N ha^{-1} to mimic nutrient status of a typical turfgrass putting green. The lysimeters were mowed twice weekly at 0.3175 cm with clippings removed. Treatments commenced in July and continued with weekly foliar and root applied urea N resulting in a total of 1.09 kg of total N for the 12 week study.

Urea N Treatments

For the foliar treatment 18.91 grams of urea N was dissolved in 500 ml of water and 273. 7 ml was sprayed over the lysimeters with a CO_2 pressurized backpack sprayer with a TeeJet 8002 VS nozzle. For the root application of urea N 1.95 grams of urea (Fisher Scientific) was dissolved in 1000 ml of water. Each lysimeter received 20 ml of the urea solution delivered via 60 ml syringe into the root zone to ensure that no fertilizer was absorbed by the foliage. Hoagland's solution derived from Hoagland and Arnon (1950) was applied twice (6 and 10 weeks) during the duration of the study (Table 3.2)

Saline and Fresh Water Irrigation

Salinity irrigation and fresh water irrigation volumes were obtained gravimetrically. Saline irrigation water was comprised of 160 grams NaCl (Fisher Scientific) dissolved in 20 L of water delivering 8,000 ppm NaCl. 25% of the available volume in the lysimeter was flushed using saline or fresh irrigation at a volume of 3.295 L at the beginning of the experiment.

Data Collection, Processing, Analysis

Parameters measured included: turf quality (TQ), leaf tissue nutrient analysis, electrolyte leakage (EL), and proline accumulation. Visual TQ was rated weekly based on color, density, texture, and uniformity of the turfgrass surface. Quality was evaluated from 1 to 9, 1 = brown, dead turfgrass, 6 = minimal acceptable turfgrass, 9 = ideal green, healthy turfgrass.

Tissue Nutrient Concentration Assay

Tissue N analysis was done by combustion utilizing a LECO FP528 N combustion analyzer (St. Joseph, MI and mineral analysis of leaf tissue for P, K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, Fe, and S by HNO_3/H_2O_2 digestion, then ICP. For leaf tissue Na^+ content, Weigh 1.0 g sample into a 150 mL beaker. Add 100 mL H_2O and place on stirrer for 30 min. Filter mixture with metal filter and pour filtrate into a large test tube. Analysis was conducted using ICP-mass spectrometry.

Electrolyte Leakage Assay

Leaf electrolyte leakage (EL) was measured to evaluate cell membrane stability. For EL analysis, 0.2-0.5 g fresh leaf tissue was harvested from each lysimeter and put in a 50 ml centrifuge tube and kept on ice. Next, Millipore water was used to rinse the clippings to ensure excess salt and fertilizer residue was removed. Following the rinse, 20 milliliters of Millipore water was added weighed and the tubes were incubated for 16-24 hours at 4 ° C. The initial conductance (Ci) was recorded using a conductance meter (AB30, Fisher Scientific, USA.) after incubation. Next, the leaf tissue was autoclaved for 50 minutes. Millipore water was added up to the original volume before the autoclave

and the conductance of the incubated solution and the killed tissue was taken (Cmax). Relative EL was calculated as (Ci/Cmax) *100.

Proline Content Assay

Proline was extracted from 100 mg of leaf tissue and determined spectrophotometrically at 520 nm according to Bates (1973).

Data Analysis

All data were analyzed by JMP 9.0 (SAS Institute Cary, NC). Means were separated by Fisher's LSD. There appeared to be unusually large variation in the data. To determine if the large variation could be attributed to a few unusual observations, an analysis of the analysis model residuals was preformed. A box-plot (Tukey, Exploratory Data Analysis, 1971) was constructed from the residuals and any residuals that were unusually large or small were indicated on the box-plot as outliers. The outliers were investigated to ensure they were not simply data entry errors. If the data were accurate and an outlier, the observations associated with the outlier residuals were removed from the data set, and the analysis repeated. If the analysis produced similar results, the large variation was considered simply part of the experiment and not due to a few unusual observations. If the analysis results changed, then the removal of the unusual observations was considered and important step in finding the true impact of the experimental factors.

Results

Turf Quality

Turf quality (TQ) was influenced by the main effects of irrigation regime and genotype. Overall averages of TQ were 5.98 and 7.05 for saline and fresh water irrigation respectively. Saline irrigation reduced the mean TQ to under acceptable levels, whereas overall TQ means for fresh water remained above 6. The main effect of genotype exhibited differences in turf quality with Diamond and 'Mini-Verde' exhibiting the highest overall means at 6.87 and 6.71 respectively. Seadwarf and TifEagle exhibited similar turf qualities at 6.47 and 6.49 respectively. Champion had the lowest TQ mean at 6.04. Over the course of the twelve week study TQ fluctuated due to salinity stress, genotype, and rating date. Fluctuations in TQ over the course of the study can be visualized in Figure 3.2 which displays TQ over 12 rating dates as influenced by genotype under salinity stress and Figure 3.1 under fresh water irrigation. Figure 3.3 also demonstrates the reduction in TQ caused by salinity stress at the conclusion of the study.

N concentration

At six weeks, N content in the leaf tissue was not affected by any main effect; however, there was a significant increase in N content in 2010 of the study (2.14 and 2.46 %DW respectively) (Table 3.3). A significant fertilizer regime*genotype interaction occurred. Root applications of urea N resulted in significantly higher total N concentration in Diamond and MiniVerde, whereas Seadwarf responded with higher N concentrations from foliar applications. TifEagle and Champion didn't exhibit a difference in N concentration for either fertility regime. Under salinity stress, Seadwarf

benefited from foliar fertilization which resulted in significantly higher concentrations of N in the leaf tissue at 2.40 % DW compared to 1.96% DW under root applications of urea. This was the only genotype that benefited from foliar fertilization under salinity stress. MiniVerde and TifEagle exhibited significantly higher N concentrations through root applications of urea under salinity stress, while the other genotypes did not show a preference for either fertility regime. Under fresh water irrigation, Diamond and MiniVerde displayed a greater N concentration in leaf tissue under root applications of urea nitrogen. Under fresh water irrigation, TifEagle was the only genotype at six weeks to display greater N concentration in leaf tissue under foliar fertility while Seadwarf and Champion didn't display change in N concentration under either fertility regime.

After 12 weeks, the main effect of irrigation regime was significant. Resulting in significantly higher leaf tissue N concentration, 2.63 % DW under fresh water treatments, compared to 2.44 % DW under salinity stress. At the conclusion of the study run 2 displayed greater overall N concentrations in leaf tissue (2.66%DW) than run 1 at (2.43 % DW). At 12 weeks an irrigation regime*genotype interaction was significant. Reduced N concentrations in leaf tissue were displayed in Diamond and Seadwarf under salinity stress, while the three ultradwarf bermudagrasses had similar N concentrations under either irrigation regime. Under fresh water treatments, Diamond and MiniVerde displayed significantly higher N concentrations under root applications of urea N. TifEagle was the only genotype to exhibit higher N leaf tissue concentration under foliar fertility and fresh water irrigation. Under salinity stress, Seadwarf was the only genotype to display higher N concentrations in leaf tissue from foliar applications of urea N. All

other genotypes exhibited similar N concentrations under salinity stress regardless of fertility regime. There was a significant irrigation regime*run interaction, indicating that in run 2 fresh water irrigation resulted in higher tissue N concentrations than salinity irrigation water. In run 1, both fresh and saline irrigation water resulted in similar N concentrations in leaf tissue. Lastly, in run 1, overall tissue N concentration was not significantly different in both regimes. However, in run 2, there was a significant difference in treatment effects on tissue N with root applications of urea resulted in significantly higher leaf tissue N concentrations.

Phosphorus Concentration

At six weeks, phosphorus (P) concentrations within the leaf tissue were affected significantly by irrigation regime, with fresh water irrigation resulting in higher P levels (Table 3.4). Fresh water irrigation resulted in 0.26 % DW, whereas saline irrigation reduced leaf P concentrations to 0.23% DW. Upon closer examination, an irrigation regime*genotype interaction occurred. All genotypes exhibited higher P levels under fresh water irrigation except Champion and TifEagle which didn't show a difference in P concentration under either irrigation regime. An irrigation regime*fertilizer regime*genotype interaction was also significant. Under salinity irrigation, the only genotype to display greater P concentrations from foliar applications of urea was Seadwarf with a significant increase in P content from 0.18 % DW under root applications of urea to 0.26% DW for foliar applications. However, under fresh water irrigation, Seadwarf didn't exhibit a preference for either fertility regime, showing similar concentrations under each regime. TifEagle displayed higher P concentration in leaf

tissue under foliar applications of urea under fresh water irrigation, while Diamond demonstrated greater P concentrations under root fertility.

At 12 weeks there was a significant main effect of irrigation regime with a decrease in P leaf tissue content under salinity stress from 0.29 % DW to 0.24 % DW. However, an irrigation regime*genotype interaction was significant with fresh water irrigation resulting in higher leaf P concentration in all genotypes except Champion. Similar to the six week results of the study, an irrigation*fertilizer regime*genotype interaction was exhibited. The only genotype to benefit from foliar urea N applications under salinity stress was Seadwarf that displayed an increase in P content over root applications (0.20 %DW, and 0.26% DW respectively). At the conclusion of the study, data displayed that during run 2 root applications of urea resulted in significantly higher levels of P in the leaf tissue, whereas in run 1 the overall P content was not significantly different for either fertility regime.

Potassium Concentration

At the halfway point of the study, there was a significant irrigation regime main effect on K^+ concentration in the leaf tissue. Fresh water irrigation resulted in significantly higher levels of K^+ than salinity irrigation, 1.52 % DW and 1.16% DW respectively (Table 3.5). Genotype effects and an interaction of genotype*irrigation regime were also significant. At six weeks, TifEagle displayed the highest K^+ concentration in the leaf tissue. Champion exhibited the greatest decrease in K^+ due to salinity stress at the halfway point of the study. K^+ concentration in Champion leaf tissue was 1.89 % DW under fresh water irrigation, whereas saline irrigation resulted in K^+

concentrations of less than 1 % DW. All other genotypes displayed similar K^+ concentrations in leaf tissue under both irrigation regimes.

At 12 weeks, an irrigation main effect was exhibited with saline irrigation significantly reducing overall tissue K^+ concentration in leaf tissue from 1.72 % DW to 1.15 % K^+ DW. A significant genotype main effect was exhibited with TifEagle and Champion displaying the most elevated levels of K^+ in the leaf tissue, while MiniVerde, Diamond and Seadwarf all had similar K^+ concentrations in leaf tissue (Table 3.5). A highly significant irrigation regime*genotype interaction was exhibited. Under saline irrigation treatments levels of K^+ in leaf tissue significantly reduced in Champion, Diamond, MiniVerde and Seadwarf. The largest decrease in K^+ concentration in leaf tissue was displayed in Champion with 2.38 %DW under control treatments, and less than 1 % DW under salinity stress. TifEagle was the only genotype that didn't exhibit significantly decreased K^+ concentrations under salinity stress (1.79 and 1.50 % DW respectively for fresh and saline irrigation water). An irrigation regime*fertilizer regime*genotype interaction was significant. Under fresh water irrigation, all genotypes displayed similar K^+ leaf concentrations under each fertilizer regime. However, under salinity stress, MiniVerde displayed higher K^+ concentration under root applications of urea N, while TifEagle exhibited higher K^+ concentration in leaf tissue under foliar fertility. All other genotypes displayed similar leaf K^+ concentrations under salinity irrigation regardless of fertility regime.

Sodium Concentration

At six weeks, irrigation regime, fertility regime, and genotype were significant in the Na^+ content of the leaf tissue (Table 3.8). Under salinity irrigation, Na^+ levels in the leaf tissue reached 1.82 %, whereas fresh water irrigation only accumulated 0.26 % DW Na^+. Root applications of urea N resulted in higher Na^+ concentrations at 1.10 % DW in leaf tissue compared to foliar treatments at 0.99 % DW. A highly significant interaction of irrigation regime*genotype occurred (Figure 3.5). A highly significant irrigation regime*fertilizer regime*genotype interaction was exhibited. Under salinity stress, Diamond and TifEagle displayed significantly higher Na^+ concentrations in leaf tissue under root applications of urea N. Champion and MiniVerde didn't show a difference in Na^+ content for either fertility regime under salinity stress. Seadwarf exhibited significantly greater Na^+ concentration in leaf tissue under foliar fertility than root fertilization at 1.92 and 1.21 % Na^+ respectively. All genotypes exhibited similar Na^+ concentrations in leaf tissue under fresh water irrigation regardless of the fertility regime. Overall levels of Na^+ concentration in leaf tissue were higher in run 2 at 1.10 % DW than run 1 at 0.98 % DW.

At 12 weeks, there were significant main effects for irrigation regime, fertilizer regime, and cultivar in the Na^+ concentration of the leaf tissue (Table 3.8). An increase in Na^+ concentration in leaf tissue under saline irrigation accounted for an increase of 625% (0.32 % DW to 2.0 % DW). Under root urea N fertility plants exhibited higher Na^+ contents compared to foliar urea fertility at 1.29 % DW and 1.03 % DW respectively. Significant interactions were also exhibited. A highly significant irrigation regime*fertilizer regime occurred. Under saline irrigation, root applications of urea N

resulted in significantly higher Na^{2+} concentration in leaf tissue than foliar applications (2.22 % and 1.81 %). Overall levels of Na^{+} concentration under control conditions did not differ between fertility regimes. Under salinity stress, Diamond exhibited the highest levels of Na^{2+} in leaf tissue at 2.46 % DW. Secondly, a fertility regime*genotype interaction significantly affected the Na^{+} concentration of leaf tissue. Root applications of urea N resulted in significantly higher Na^{2+} concentrations in Champion, Diamond, and TifEagle while 'Seadwarf was the only genotype to exhibit higher Na^{+} concentration in leaf tissue under the foliar fertility regime. Lastly, a highly significant irrigation regime*fertilizer regime*genotype occurred. Under fresh water irrigation, all genotypes regardless of fertility regime, exhibited similar Na^{+} concentrations in the leaf tissue. However, under salinity stress, Champion, Diamond and TifEagle exhibited greater Na^{+} concentrations under root applications of urea, while 'Seashore' exhibited higher Na^{+} concentration under foliar fertility.

Calcium Concentration

Overall calcium levels in the leaf tissue were significantly reduced by saline irrigation water treatments. Under fresh water irrigation Ca^{2+} concentration in leaf tissue was 0.27% DW, and was reduced to 0.19% DW under salinity stress. In run 2 (0.24%DW), overall calcium concentration in leaf tissue was greater than run 1 (0.22%DW). An irrigation regime*fertilizer regime*genotype interaction was significant. Under salinity irrigation, Seadwarf exhibited significantly higher Ca^{2+} concentration in leaf tissue under foliar urea N fertility while all other genotypes demonstrated similar concentrations under either fertility regime.

At the conclusion of the study, overall levels of Ca^{2+} in the leaf tissue were reduced by saline irrigation, from 0.30 % to 0.18 % Ca^{2+}/DW. An irrigation regime*genotype interaction was significant. All genotypes displayed reduced Ca^{2+} concentrations in leaf tissue under salinity stress. Secondly, a fertilizer regime*genotype interaction was exhibited. Foliar applications of urea N resulted in higher Ca^{2+} concentration in leaf tissue of TifEagle, all other genotypes displayed similar concentrations under either fertility regime. Overall Ca^{2+} levels in leaf tissue were higher in run 2 than run 1, 0.29 and 0.19 %DW correspondingly.

Proline Accumulation

At six weeks, there was a significant irrigation regime treatment effect on proline accumulation. Overall proline accumulation increased from 0.55 μg^{-1}g FW to 2.98 μg^{-1}g FW under fresh and saline irrigation regimes respectively (Table 3.7). An interaction of irrigation regime*fertilizer regime*genotype displayed that both fertility regimes led to similar proline accumulations across all genotypes under fresh water irrigation. However, under salinity stress, Diamond accumulated the most proline of all genotypes under root applications while Seadwarf accumulated higher proline amounts with foliar applications of urea N.

At the conclusion of the study, an irrigation main effect was displayed with salinity stress increasing overall proline levels from 1.07 μg^{-1}g FW in fresh water treatments to 6.18 μg^{-1}g FW in saline irrigation treatments. A significant genotype main effect was exhibited at 12 weeks with Champion accumulating the highest levels of proline at 4.92 μg^{-1}g FW (Table 3.7). An irrigation regime*fertilizer regime was

significant at 12 weeks. Under salinity irrigation, plants receiving root applications of urea accumulated more proline at 6.64 $\mu g^{-1}g$ FW than plants receiving foliar fertility at 5.02 $\mu g^{-1}g$ FW. An irrigation regime*genotype interaction was significant at the conclusion of the study. All genotypes exhibited significantly higher levels of proline under salinity stress, with Champion displaying the greatest difference in accumulation from > 1 $\mu g^{-1}g$ FW under fresh water irrigation to over 9 $\mu g^{-1}g$ FW under salinity stress. At 9.27 $\mu g^{-1}g$ FW, Champion accumulated significantly higher proline than any other genotype under salinity stress. A fertilizer regime*genotype interaction was significant. Diamond MiniVerde and TifEagle all displayed higher proline accumulation under root fertility, and Seadwarf accumulating significantly higher proline under foliar fertility treatments. Lastly, an irrigation regime*fertilizer regime*genotype interaction was displayed. Under fresh water irrigation treatments, all combinations of genotypes and fertility regimes resulted in similar proline accumulations (Table 3.7). However, this trend was not observed under salinity stress, where a diverse range of responses was exhibited. Diamond MiniVerde and TifEagle accumulated higher levels of proline under root applications of urea N. The most salt tolerant genotype, Seadwarf was the only grass to exhibit higher proline accumulation under foliar applications of N. Overall levels of proline increased from 2.27 $\mu g^{-1}g$ FW in run 1 to 4.53 μg^{-1} g FW in run 2. A relationship was exhibited between Na^+ concentration in leaf tissue and the concentration of proline. Each genotype was analyzed at the conclusion of the study and regression analyses were conducted (Figures 3.7-3.12). Significant relationships were exhibited when increasing Na^+ concentration in leaf tissue resulted in elevated proline levels. Similar increases in

Na^+ concentration and proline accumulation were exhibited in barley leaves by Buhl and Stewart (1983).

Electrolyte Leakage

At six weeks of the study saline irrigation resulted in a 2 fold increase in EL from 17.51 % in fresh water treatments to 33.56 % under salinity stress (Table 3.6). An irrigation regime*genotype interaction was significant with all genotypes except TifEagle displaying higher EL levels under salinity stress. A significant fertilizer regime*genotype interaction was displayed. Foliar applications resulted in higher EL levels in MiniVerde and Seadwarf whereas TifEagle displayed higher EL levels under the root fertility regime. Lastly, an irrigation regime*fertilizer regime*genotype was exhibited. Under fresh water irrigation treatments, Champion displayed higher EL levels under foliar fertilization, while TifEagle exhibited higher EL under root fertility of urea N. Under saline irrigation treatments, Diamond, MinVerde, and Seadwarf displayed higher EL levels under foliar fertility of urea N. TifEagle was the only genotype under salinity stress that exhibited lower EL levels under foliar fertility treatments, a decrease from 33.99% to 20.31%.

At the conclusion of the study, there was a highly significant effect of EL due to the irrigation regime. Saline irrigation significantly increased overall EL to 39.59% from 18.59% in fresh irrigation water. Irrigation regime*genotype interactions displayed that under salinity irrigation, Champion exhibited significantly higher levels of EL than TifEagle (47.77% and 36.47% respectively). Under fresh water irrigation, all genotypes displayed similar EL levels. An irrigation regime*fertilizer regime*genotype interaction

was significant. Under salinity stress Diamond exhibited significantly higher EL readings with root applications of urea. MiniVerde displayed higher EL readings under foliar applications of urea N (48.69% and 37.13% respectively). Champion, Seadwarf and TifEagle had similar EL readings under either fertility regime. The data from run 2 had overall significantly higher EL at 33.90% compared to run 1 at 23.85%.

Discussion

At six weeks of the study, saline stress had no effect on urea N uptake which disagrees with observations by Aslam et al. (1984) who observed reduced N uptake of barley under salinity stress within hours of exposure. A diverse response among genotypes was displayed under various irrigation and fertility regimes. The only genotype to display greater N concentrations from foliar fertility under salinity stress was Seadwarf. Under salinity stress, Seadwarf displayed a 22% increase N concentration by DW in leaf tissue with foliar urea fertility. The opposite response was exhibited for MiniVerde and TifEagle under salinity stress, where root applications of urea resulted in higher N concentrations in leaf tissue. This trend was not observed for the fresh water treatments where TifEagle was the only genotype at six weeks to display higher N concentration under foliar fertility, while Diamond and MiniVerde displayed higher N concentration in leaf tissue under root applications. Seadwarf and Champion didn't display change in N concentrations under either fertility regime.

At the conclusion of the study, saline irrigation significantly reduced overall leaf tissue N concentration. This result agrees with findings by Bowman et al. (2006) in tall fescue, Hawkins et al. (1993) in wheat, Pessarakli and Tucker (1988) and Flores et al.

(2001) in tomato that showed reduced N uptake under salinity stress. There also were diverse results among genotype and irrigation regimes. At the conclusion of the study, Diamond and Seadwarf displayed lower N concentrations in their leaf tissue due to salinity irrigation, while the ultradwarf bermudagrasses exhibited similar N levels under either regime. Although Diamond and Seadwarf are considered the most salinity tolerant of the genotypes tested, their N concentrations were significantly reduced by saline irrigation, unlike the ultradwarf bermudagrasses genotypes. At 2.39 % DW and 2.28% DW in Diamond and Seadwarf' respectively, the N concentrations under salinity are within the commonly considered sufficiency range for turfgrasses (Carrow et al., 2001).

Overall concentrations of P in leaf tissue were reduced at the six weeks, from 0.27 % DW in fresh water to 0.24% DW in saline treatments. At six weeks, P levels were affected significantly by salinity and all genotypes exhibited higher P concentrations under fresh water irrigation with the exception of Champion and TifEagle, which displayed similar levels under both irrigation regimes. However, at the end of the study, only Champion exhibited the same P levels in leaf tissue under either irrigation regime. At six weeks, and also at the conclusion of the study, Seadwarf, the most salinity tolerant genotype tested benefited from foliar applications of urea N under salinity by exhibiting higher P concentrations in leaf tissue at 0.26%DW. Under root fertility treatments, the P concentration of leaf tissue in Seadwarf fell to 0.18% DW, which is considered deficient by Carrow et al. (2001). However, under fresh water irrigation, Seadwarf didn't exhibit a preference for either fertility regime, showing similar concentration in leaf tissue under each regime. TifEagle displayed greater P

concentration under foliar applications of urea under fresh water irrigation, while Diamond demonstrated greater P levels under root fertility.

Salinity stress and P nutrition of plants is a complex interaction dependent on several factors including: genotype, plant age, and composition and level of salinity (Grattan and Grieve, 1999). This is evident in the results from this study, with many conclusions being drawn from salinity stress and nutritional status of each genotype examined. Champion exhibited similar P concentration in leaf tissue under either irrigation regime while all other genotypes P levels were reduced under salinity stress. A reduction of P concentrations in plant tissue is common under salinity stress; however, there wasn't evidence of P deficiency in any of the genotypes tested.

Maintaining adequate levels of K^+ is critical for plant survival in saline environments (Grattan and Grieve, 1999). At the midpoint of the study overall K^+ concentrations within leaf tissue decreased to 1.18 % DW due to saline irrigation, which is below the common sufficiency range in turfgrasses. Of all genotypes tested, concentrations of K^+ in the leaf tissue of Champion were reduced in the greatest amount by saline irrigation at the conclusion of the study. Under fresh water irrigation treatments, concentrations of K^+ in Champion were sufficient in leaf tissue at 2.38 %DW, whereas saline irrigation reduced K^+ concentrations less than 1 % DW. This response was also seen at six weeks in the study. Under salinity stress, levels of K^+ in leaf tissue were significantly reduced in Champion, Diamond, MiniVerde, and Seadwarf. TifEagle was the only genotype not to exhibit reduced K^+ concentrations under salinity treatments at 12 weeks.

Potassium is an inorganic solute that is involved in osmoregulation by enhancing water transport in the xylem and maintaining high cell turgor pressure (Carrow et al., 2001). A decrease in K^+ uptake under salinity stress can lead to higher concentrations of Na^+ in the vacuole. leading to decreases in critical K^+ metabolic roles. An increased level of Na^+ not only interferes with K^+ uptake by the roots, but it may also disrupt the integrity of root membranes and alter their selectivity (Grattan and Grieve, 1999).

Turfgrasses grown in salt affected areas are commonly supplemented with additional Ca^{2+} fertility. However, with elevated Na^+ or Ca^{2+} levels, turfgrasses often require K^+ in greater quantities than similar non-salt affected site, particularly when frequent leaching of salts is conducted (Carrow et al., 2001). Ca^{2+} influences the K^+/Na^+ selectivity by shifting the uptake ratio in favor of K^+ at the expense of Na^+ due to enhanced membrane integrity and reduction of K^+ leakage from cells (Grattan and Grieve, 1999). By the end of the study, overall levels of Ca^{2+} in leaf tissue were reduced to deficiency levels at 0.18 % DW (Carrow et al., 2001). This reduction in leaf tissue Ca^{2+} under salinity stress is expected because the uptake of Ca^{2+} is reduced due to ion interactions, precipitation, and increases in ionic strength. All of these influences decrease the availability of Ca^{2+} to the plant (Grattan and Grieve, 1999). Maintaining an adequate concentration of calcium in salt stressed environments is an important aspect in controlling the severity of ion toxicities. All genotypes experienced a significant reduction in Ca^{2+} concentration in leaf tissue under salinity irrigation. An irrigation regime*fertility regime*genotype interaction was displayed with MiniVerde responding with higher Ca^{2+} concentration under salinity stress with root applications of urea N. As

the salt concentration in the root zone increases, plant requirement for calcium also increases. Elevated levels of Na^+ replace Ca^{2+} at cell membranes, especially root cells. Calcium displacement causes loss of membrane integrity, leading to leakage of nutrients and cell lysis as indicated by EL. Calcium deficiency has been reported to enhance Pythium blight (*Pythium* spp.) and other disease susceptibility (Carrow et al., 2001). Calcium plays an essential role in processes that preserve the structural and functional integrity of plant membranes, stabilize cell wall structures, regulate ion transport and selectivity, and control ion exchange behavior as well as cell wall enzyme activities (Grattan and Grieve, 1999).

By six weeks of the study, overall concentrations of Na^{2+} in leaf tissue increased due to irrigation regime. However, there were diverse results between genotype's ability to exclude Na^+ ion uptake in the root zone. Under saline irrigation, Seadwarf (1.57 % Na^+) and TifEagle (1.64 % Na^+) exhibited significantly lowered levels of Na^+ in their leaf tissue compared to the other species. Champion, Diamond, and MiniVerde averaged 2.01 % Na^+ within leaf tissue. Further interactions were displayed under salinity stress for genotype and fertility regimes. Often relations between salinity and mineral nutrient of horticultural crops are extremely complex (Grattan and Grieve, 1999). Under fresh water irrigation, all genotype displayed similar Na^+ concentrations in leaf tissue regardless of fertility regime. This was not displayed under salinity stress. Diamond and TifEagle exhibited significantly higher Na^+ concentrations in leaf tissue under root applications of urea nitrogen, while Seadwarf exhibited significantly higher

Na^+ concentration in leaf tissue under foliar fertility than root fertilization at 1.92 and 1.21 % Na^+ respectively.

By 12 weeks an overall increase in Na^+ within the leaf tissue under saline irrigation accounting for 646% increase (0.32 to 2.10 %). A fertility regime main effect was displayed also that resulted in higher overall Na^{2+} concentration in leaf tissue under root urea N fertility than foliar application at1.29 and 1.13 % Na^+ respectively. At the conclusion of the study, many interactions between salinity and fertility regimes were displayed. Under salinity stress, root applications of urea N resulted in significantly higher overall Na^{2+} concentrations in leaf tissue than foliar applications (2.25 % and 1.94 %). Overall levels of Na^+ concentration in leaf tissue under control conditions did not differ between fertility regimes. Under fresh water irrigation, all genotypes regardless of fertility regime, exhibited similar Na^+ concentrations in the leaf tissue. However, under salinity stress, Champion, Diamond and TifEagle exhibited greater Na^+ concentrations under root applications of urea, while 'Seashore' exhibited greater Na^+ concentration under foliar fertility.

Studies focusing on salinity and its influence on mineral nutrition haven't examined foliar and root absorbed N treatments. Plants can respond to salinity by excluding the salt ions or by including them. Under each response there are adaptations to achieve salt tolerance. In excluders this includes: increased synthesis of organic solutes and a decrease in surface area (arid climates/succulents). The ion includers rely on salt compartmentalization, synthesis of compatible solutes, salt excretion, leaf drop and re-translocation in phloem (Marschner, 1995).

Halophytic grasses that are ion excluders rely on osmoptrotectants to maintain turgor. These compatible osmolytes are organic compounds such as glycine betaine, proline and to a lesser extent K^+ and Ca^{2+} (Lee et al., 2007). These two mechanisms for salinity stress have been explored by Marcum and Pessarakli (2006) with exclusion and excretion being the most effective methods. Marcum and Murdoch (1994) found that salinity tolerance of C_4 turfgrasses is related to Na^+ and Cl^- restriction from shoots. Although we found significantly higher levels of Na^+ in the shoot tissue, the genotype examined most likely compartmentalized the saline ions into the vacuole.

At six weeks of the study, saline irrigation significantly affected overall proline accumulation in leaf tissue. An increase from 0.55 μg^{-1}g FW to 2.92 μg^{-1}g FW under fresh and saline irrigation regimes respectively was displayed. Champion, Diamond and MiniVerde exhibited higher proline accumulation under saline irrigation regime, while proline accumulation for Seadwarf and TifEagle was similar for either irrigation regime. An interaction of irrigation regime*fertilizer regime*genotype showed that both fertility regimes led to similar proline accumulations across all genotypes under fresh water irrigation. However, under salinity stress, Diamond accumulated more proline under root applications while Seadwarf accumulated higher proline amounts with foliar applications of urea N. Further examination of osmoprotectant accumulation and Na^+ concentration needs to be conducted in warm-season turfgrass management. Determination of Na^+ threshold levels in leaf tissue could lead to a better understanding of salinity tolerance and precision turfgrass management in salt affected environments.

Electrolyte leakage (EL) was significantly affected by irrigation regime at six and twelve weeks of the study. This plant response was expected due to the increase in saline ions in the root-zone, leading to a deficiency in calcium. This deficiency leads to a breakdown in the integrity of cell walls and membranes. Saline irrigation significantly increased overall EL to 44.48% from 18.87% in fresh irrigation water. A significant genotype effect was seen with Champion exhibiting significantly higher EL (34.14%) than TifEagle (28.50%). All other genotypes had similar relative EL levels. Irrigation regime*genotype interactions showed that under salinity irrigation, Champion exhibited significantly higher levels of EL than TifEagle (47.77% and 36.47% respectively). Under fresh water irrigation, all genotypes had similar EL levels. Under salinity stress Diamond exhibited significantly higher EL readings with root applications of urea. MiniVerde displayed higher EL readings under foliar applications of urea N (48.69% and 37.13% respectively). Ward et al. (1986) illustrated the importance of calcium for selective ion uptake in plants under salinity stress. Through the restriction of Na^+ influx and translocation, elevated levels of calcium can protect plants from salt injury.

Conclusions

The examination of urea N delivery method has been conducted in common warm-season turfgrasses under moderate salinity stress. Foliar and root applied urea N resulted in similar N, P, and K^+ concentrations in the leaf tissue of all genotypes However, overall leaf tissue Na^+ concentrations at the conclusion of the study were significantly higher in most genotypes excluding examined, excluding Seadwarf under root fertilization than foliar applied urea-N turfgrasses. The increase in Na^+

concentration in leaf tissue due to root applied urea was not expected and could be due to soil related factors. Direct uptake of urea by roots is possible due to the evidence of urea specific transporters and aquaporins that passively transport urea on many plants (Liu et al., 2003). However, if the urea in the root-zone is hydrolyzed by urease present in the soil, a small increase in soil pH will occur (Reynolds et al., 1985). This fluctuation in soil pH due to the increased hydrolysis of urea following root applications could be driving Na^+ uptake. However, definitive conclusions of this theory cannot be made without examining soil urease activity, and pH, and determining Na^+ concentrations in root tissue. A portion of the urea derived from fertilizer applications is being hydrolyzed by soil borne urease, while there is evidence that urea can be taken up directly through urea transporters and aquaporins on the root membrane (Liu et al., 2003). Further research needs to be conducted to determine which N form is being taken up by the plant in the largest quantities following root applied urea N and the soil pH fluctuations that could possibly further explain the Na^+ uptake which was recorded in this study.

Seadwarf, the most salinity tolerant genotype examined, exhibited significant increases in N concentration under foliar urea N applications and slight improvements in TQ under moderate salinity stress. In addition, foliar applications of urea N resulted in elevated Na^+ concentration in the leaf tissue of Seadwarf at the midpoint and conclusion of the study, which was the only genotype to display such a response. At 6 weeks, Seadwarf responded to foliar urea N applications with higher P concentrations in leaf tissue, however at 12 weeks, P concentrations in leaf tissue were greater under root

applied N. Findings from this study suggest that foliar applications of urea N provide an alternative to traditional granular fertilization when root zone salinity is elevated.

Although TQ was initially reduced under salinity stress; a rebound in TQ was displayed from the midpoint until the conclusion of the study. Salt stress mechanisms including accumulation of proline could be contributing to the increase in TQ due to better salt tolerance. Marcum and Pessarakli (2006) found that TifEagle, Champion, and MiniVerde exhibited similar salt tolerances, however, results from this study suggest otherwise. Champion displayed the greatest reduction in TQ among all genotypes tested while responded to salinity stress with the greatest EL % and proline accumulations. It is possible that warm-season turfgrasses, including Seadwarf, could possibly be Na^+ deficient. Future research examining Na^+ sufficiency concentrations needs to be determined in common warm-season turfgrasses.

Due to the complexity of the interactions exhibited under salinity stress, it is commonly difficult to draw definitive conclusions on the status of mineral nutrition within turfgrasses. Multiple responses were displayed among genotypes under foliar urea N fertilization and salinity stress. Seadwarf seashore paspalum displayed interesting responses to salinity stress and fertility regime over the course of the study. Further research examining Na^+ and N transport and uptake needs to be determined in warm-season turfgrasses to make recommendations for best nutrient management practices in salt-affected areas. Urease activity in the soil and plant tissue following urea N fertilization needs to be fully examined to determine nutrient uptake and assimilation in an effort to maximize N use efficiency. Finally, Ni^{2+}nutrition and supplementation of

warm-season turfgrasses needs to be researched due to Ni^{2+}'s essential role in the hydrolysis of urea.

Table 3.1. Soil chemical properties of 85%:15% (v:v) sand: peat used for the project Clemson University Greenhouse Research Complex.

		----------kg^{-1} ha----------											----------% Base Saturation----------				
Soil pH	Buffer pH	P	K	Ca	Mg	Zn	Mn	Cu	B	Na	CEC†	Acidity†	Ca	Mg	K	Na	Total
5.93	7.95	4.8	14.9	665.9	78.4	0.7	1.8	0.3	0.1	17.5	2.2	0.4	66.6	13	1	1.6	81.6

†Meq 100 g

Table 3.2. Stock solutions and concentrations for micronutrient, NaCl, phosphorus, and potassium solutions based on Hoagland and Arnon (1950)

Nutrient	Stock Solution Concentration	Experimental Concentration
A. Micronutrient Stock	(g/L)	1ml/L
Boric Acid, H_3BO_3	2.86	
Manganese chloride, $MnCl_2$*4 H_2O	1.81	
Zinc sulfate, $ZnSO_4$ *7 H_2O	0.22	
Copper sulfate, $CuSO_4$ *5 H_2O	0.08	
Molybdic acid, MoO_3*H_2O	0.02	
B. Fe (Sequestrene)	21.0	1ml/L
C. Sodium Chloride, NaCl	250	
D. Phosphorus, P_2O_5 (0-30-0)	0.81	
E. Potassium, K_2O (0-0-25)	0.91 ml/L	
	1.90ml/L	

Table 3.3. Tissue N concentration in leaf tissue as influenced by salinity regime, fertility regime, genotype, and run in Clemson University Greenhouse Research Complex at two harvest events (6 and 12 weeks after initiation of treatments).

Main effects	6 Week	12Week
	----------% DW---------	
Salinity (S)		
Control	2.31	2.63
8,000 ppm	2.29	2.44
Fertility Regime (F)		
Foliar	2.25	2.52
Root	2.35	2.56
Genotype (G)		
Seadwarf	2.33	2.56
Diamond	2.37	2.57
TifEagle	2.22	2.51
Champion	2.24	2.44
MiniVerde	2.34	2.62
$LSD_{(0.05)}$	NS	NS
Run (R)		
1	2.14	2.43
2	2.46	2.65
	ANOVA	
Source of variation†		
S	NS	**
F	NS	NS
G	NS	NS
R	***	**
S*F	NS	NS
S*G	*	**
F*G	**	**
S*F*G	**	**
S*F*R	NS	NS
S*G*R	NS	NS
F*G*R	NS	NS
S*F*G*R	NS	NS

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Main effect and interactions including Run (R) exhibited differences in magnitude, not changes in trend.

Table 3.4. Tissue P concentration in leaf tissue as influenced by salinity regime, fertility regime, genotype, and run in Clemson University Greenhouse Research Complex at two harvest events (6 and 12 weeks after initiation of treatments).

Main effects	6 Week	12Week
	----------% DW----------	
Salinity (S)		
Control	0.26	0.29
8,000 ppm	0.23	0.24
Fertility Regime (F)		
Foliar	0.25	0.26
Root	0.25	0.27
Genotype (G)		
Seadwarf	0.25	0.27
Diamond	0.26	0.27
TifEagle	0.24	0.26
Champion	0.24	0.26
MiniVerde	0.26	0.27
$LSD_{(0.05)}$	NS	NS
Run (R)		
1	0.25	0.26
2	0.25	0.27
	ANOVA	
Source of variation†		
S	***	***
F	NS	NS
G	NS	NS
R	NS	NS
S*F	NS	NS
S*G	**	*
F*G	**	NS
S*F*G	**	**
S*F*R	NS	NS
S*G*R	NS	NS
F*G*R	NS	NS
S*F*G*R	NS	NS

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Main effect and interactions including Run (R) exhibited differences in magnitude, not changes in trend.

Table 3.5. Tissue K^+ concentration in leaf tissue as influenced by salinity regime, fertility regime, genotype, and run in Clemson University Greenhouse Research Complex at two harvest events (6 and 12 weeks after initiation of treatments).

Main effects	6 Week	12Week
	----------% DW----------	
Salinity (S)		
Control	1.52	1.72
8,000 ppm	1.16	1.15
Fertility Regime (F)		
Foliar	1.36	1.43
Root	1.33	1.43
Genotype (G)		
Seadwarf	1.20	1.27
Diamond	1.23	1.32
TifEagle	1.53	1.61
Champion	1.51	1.64
MiniVerde	1.25	1.34
$LSD_{(0.05)}$	0.23	0.23
Run (R)		
1	1.38	1.45
2	1.30	1.42
	ANOVA	
Source of variation†		
S	***	***
F	NS	NS
G	**	**
R	NS	NS
S*F	NS	NS
S*G	**	***
F*G	NS	NS
S*F*G	NS	NS
S*F*R	NS	NS
S*G*R	NS	NS
F*G*R	NS	NS
S*F*G*R	NS	NS

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Main effect and interactions including Run (R) exhibited differences in magnitude, not changes in trend.

Table 3.6. Electrolyte leakage in leaf tissue as influenced by salinity regime, fertility regime, genotype, and run in Clemson University Greenhouse Research Complex at two harvest events (6 and 12 weeks after initiation of treatments).

Main effects	6 Week	12Week
	----------%----------	
Salinity (S)		
Control	17.51	18.59
8,000 ppm	33.56	39.59
Fertility Regime (F)		
Foliar	26.48	28.92
Root	24.59	28.83
Genotype (G)		
Seadwarf	24.72	26.94
Diamond	24.32	29.70
TifEagle	22.21	24.85
Champion	29.02	32.87
MiniVerde	27.40	30.01
$LSD_{(0.05)}$	4.45	4.60
Run (R)		
1	16.72	23.85
2	34.35	33.90
	ANOVA	
Source of variation†		
S	***	***
F	NS	NS
G	*	*
R	***	***
S*F	NS	NS
S*G	***	**
F*G	***	***
S*F*G	***	**
S*F*R	NS	NS
S*G*R	**	NS
F*G*R	***	**
S*F*G*R	**	**

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Main effect and interactions including Run (R) exhibited differences in magnitude, not changes in trend.

Table 3.7. Proline accumulation in leaf tissue as influenced by salinity regime, fertility regime, genotype, and run in Clemson University Greenhouse Research Complex at two harvest events (6 and 12 weeks after initiation of treatments).

Main effects	6 Week	12Week
	---------- μg^{-1} g FW ----------	
Salinity (S)		
Control	0.55	1.07
8,000 ppm	2.98	6.18
Fertility Regime (F)		
Foliar	1.68	3.86
Root	1.85	3.40
Genotype (G)		
Seadwarf	1.25	3.00
Diamond	2.52	4.19
TifEagle	1.27	3.01
Champion	2.09	4.92
MiniVerde	1.70	3.02
$LSD_{(0.05)}$	1.04	1.17
Run (R)		
1	1.53	2.32
2	2.00	4.93
	ANOVA	
Source of variation†		
S	***	***
F	NS	NS
G	NS	**
R	NS	***
S*F	NS	NS
S*G	NS	**
F*G	*	***
S*F*G	*	***
S*F*R	NS	NS
S*G*R	NS	NS
F*G*R	NS	NS
S*F*G*R	NS	**

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Main effect and interactions including Run (R) exhibited differences in magnitude, not changes in trend.

Table 3.8. Tissue Na^+ concentration in leaf tissue as influenced by salinity regime, fertility regime, genotype, and run in Clemson University Greenhouse Research Complex at two harvest events (6 and 12 weeks after initiation of treatments).

Main effects	6 Week	12Week
	----------%DW----------	
Salinity (S)		
Control	0.26	0.32
8,000 ppm	1.82	2.00
Fertility Regime (F)		
Foliar	0.99	1.03
Root	1.10	1.29
Genotype (G)		
Seadwarf	0.94	1.18
Diamond	1.16	1.34
TifEagle	0.95	1.07
Champion	1.13	1.21
MiniVerde	1.03	0.98
$LSD_{(0.05)}$	0.11	0.19
Run (R)		
1	0.98	0.96
2	1.10	1.36
	ANOVA	
Source of variation†		
S	***	***
F	**	***
G	***	**
R	**	***
S*F	*	***
S*G	***	**
F*G	***	***
S*F*G	***	***
S*F*R	NS	NS
S*G*R	NS	NS
F*G*R	NS	*
S*F*G*R	NS	*

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Main effect and interactions including Run (R) exhibited differences in magnitude, not changes in trend.

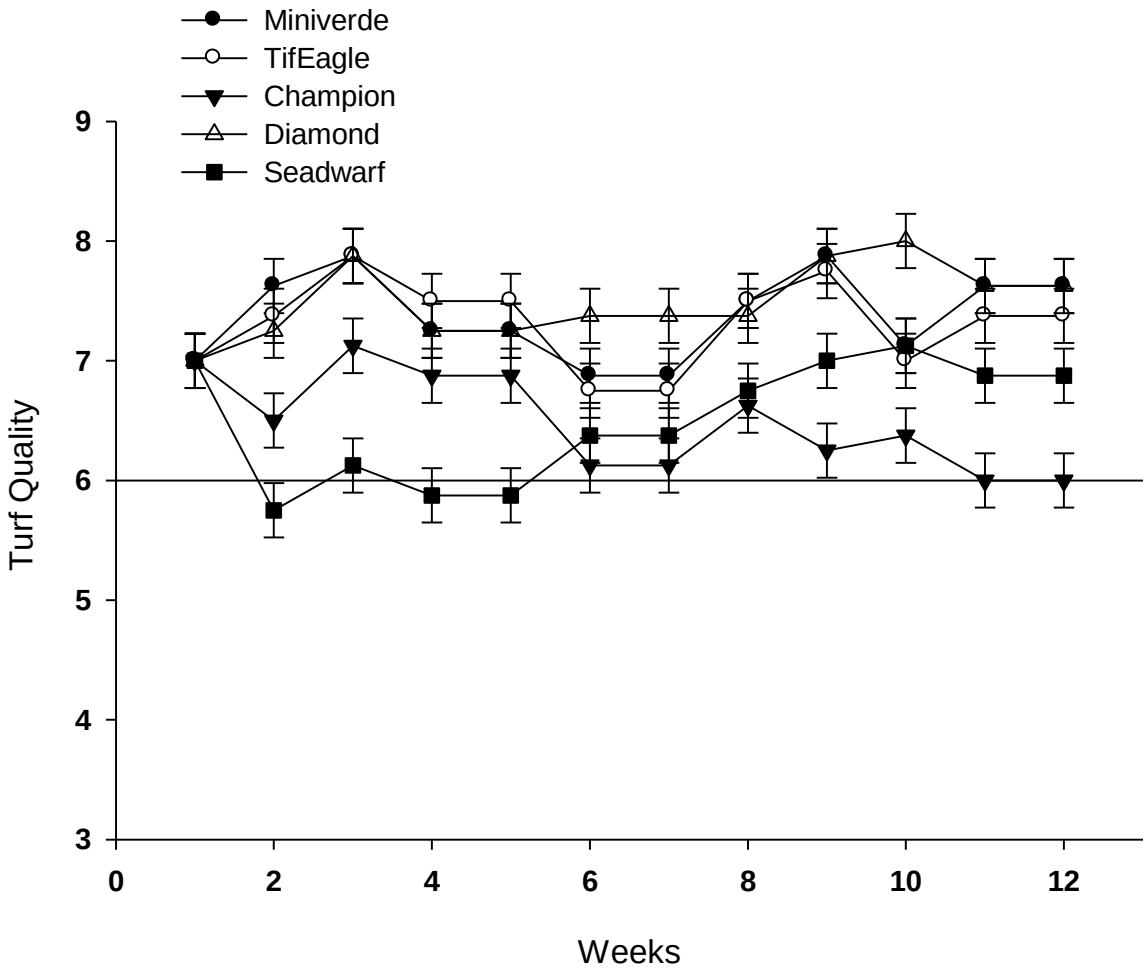


Figure 3.1. Turf quality (0-9, >6 acceptable) of MiniVerde, TifEagle, Champion, Diamond and Seadwarf under fresh water irrigation at the Clemson University Greenhouse Complex over 12rating dates. Means were separated at P≤0.05 by protected LSD.

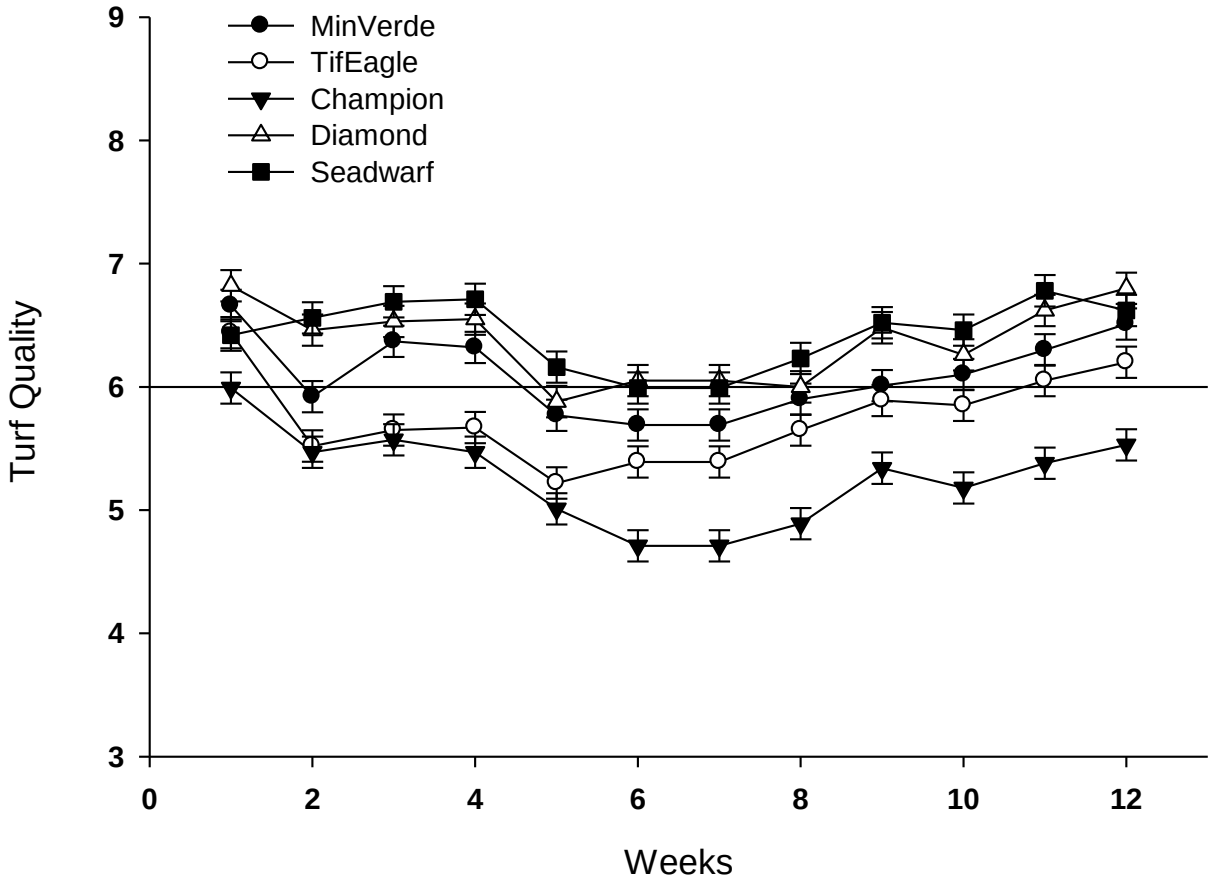


Figure 3.2. Turf quality (0-9, >6 acceptable) of MiniVerde, TifEagle, Champion, Diamond and Seadwarf under salinity stress at the Clemson University Greenhouse Complex over 12 rating dates. Means were separated at P≤0.05 by protected LSD.

Figure 3. 3. Turf quality of five warm-season turfgrass genotypes under two salinty levels (0,8,000 ppm NaCl) at the conclusion of the study.

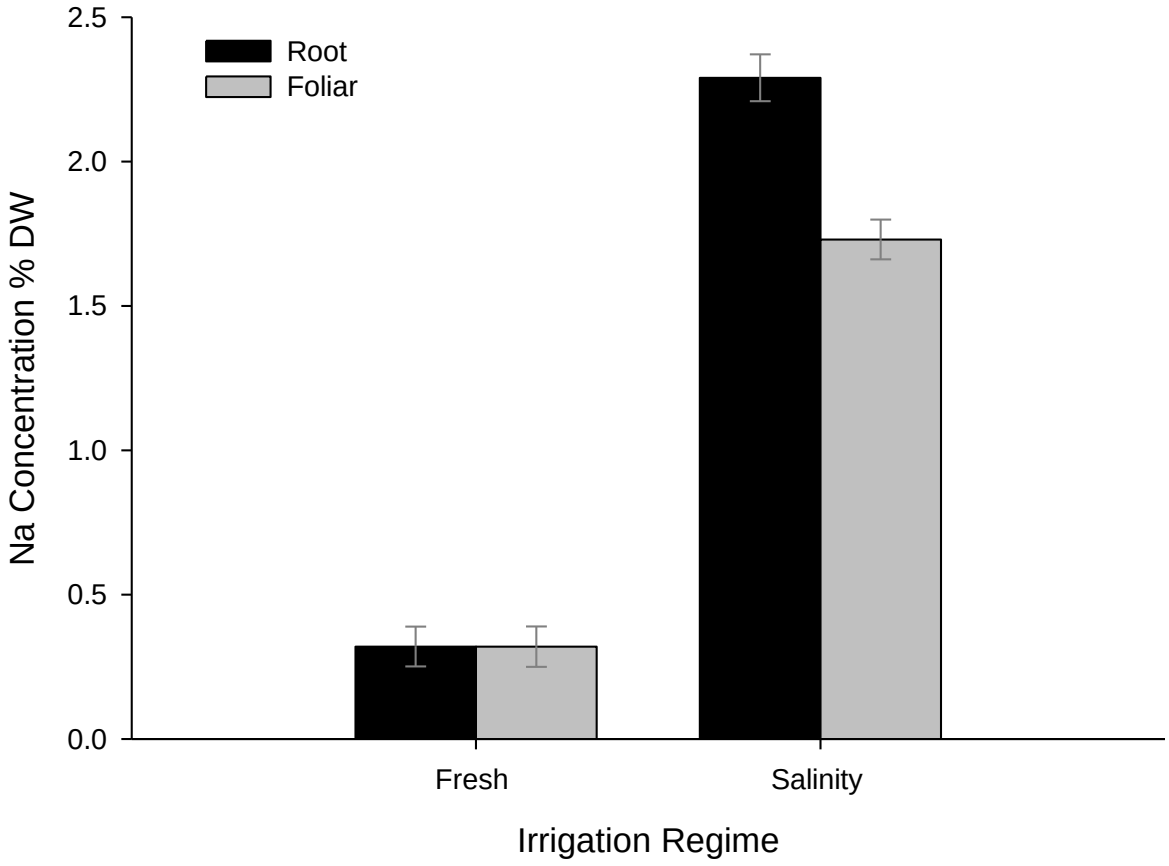


Figure. 3.4. The interaction of salinity stress and fertility regime on Na^+ concentration % DW in the leaf tissue at the conclusion of the study. Means were separated at $P \leq 0.05$ by protected LSD.

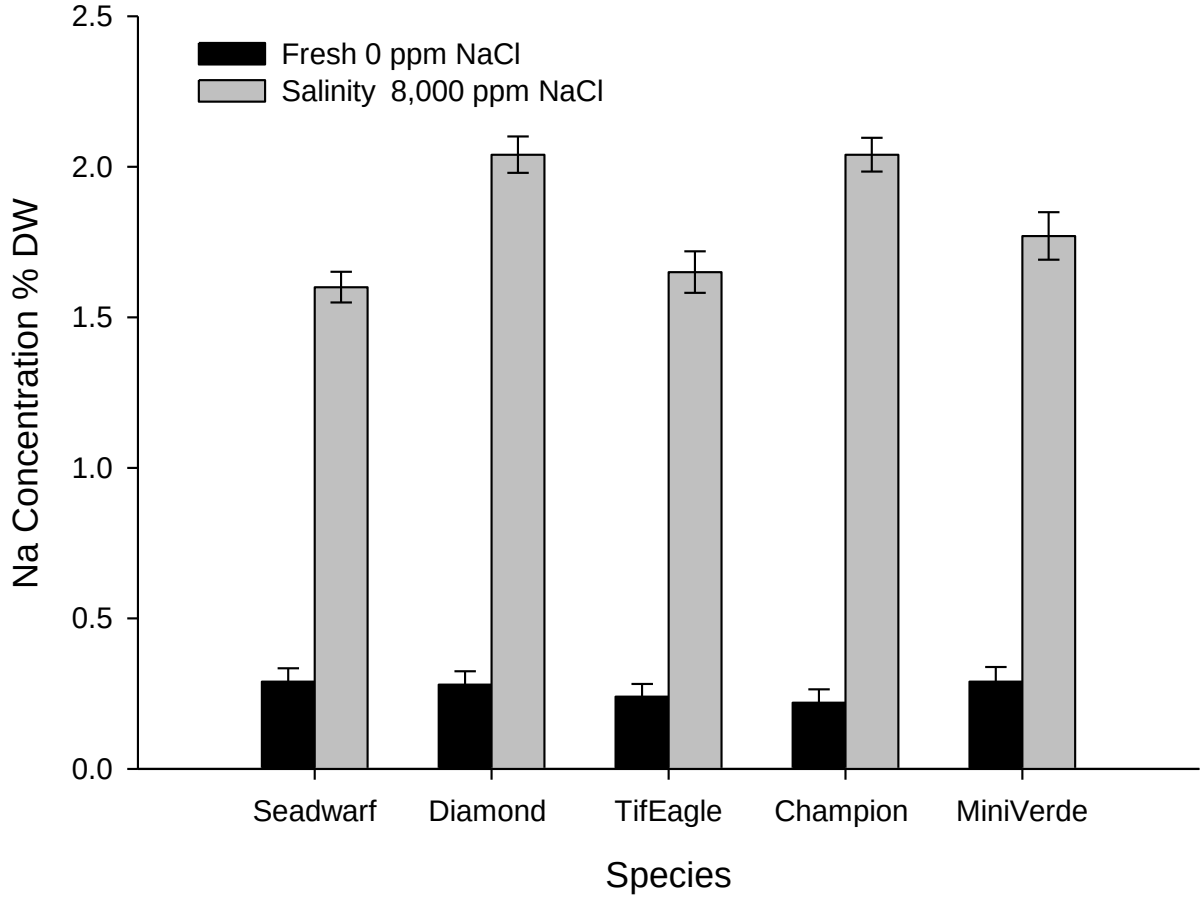


Figure 3.5. Na^+ concentration % DW of MiniVerde, TifEagle, Champion, Diamond and Seadwarf under both irrigation regimes at the Clemson University Greenhouse Complex at 6 weeks after treatments began. Means were separated at $P \leq 0.05$ by protected LSD.

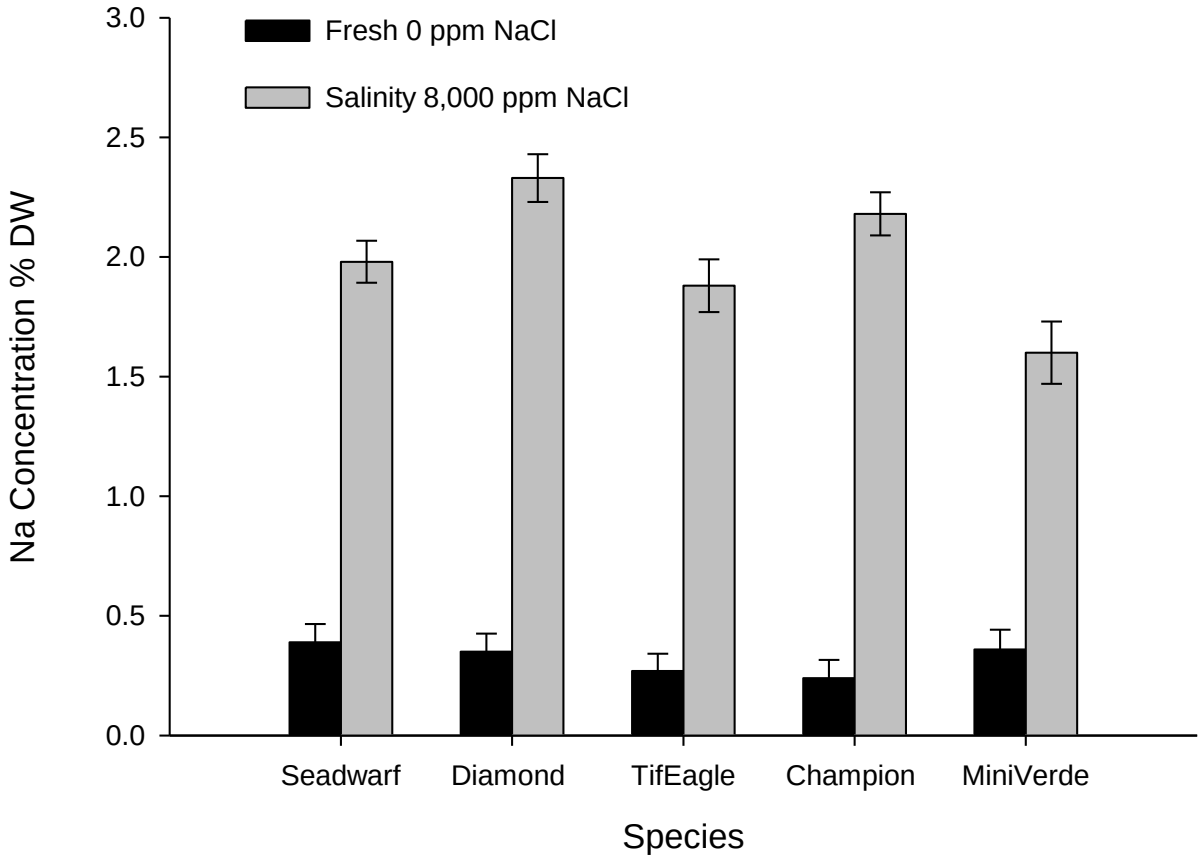


Figure 3.6. Na^+ concentration % DW of MiniVerde, TifEagle, Champion, Diamond and Seadwarf under both irrigation regimes at the Clemson University Greenhouse Complex at the conclusion of the study. Means were separated at $P \leq 0.05$ by protected LSD.

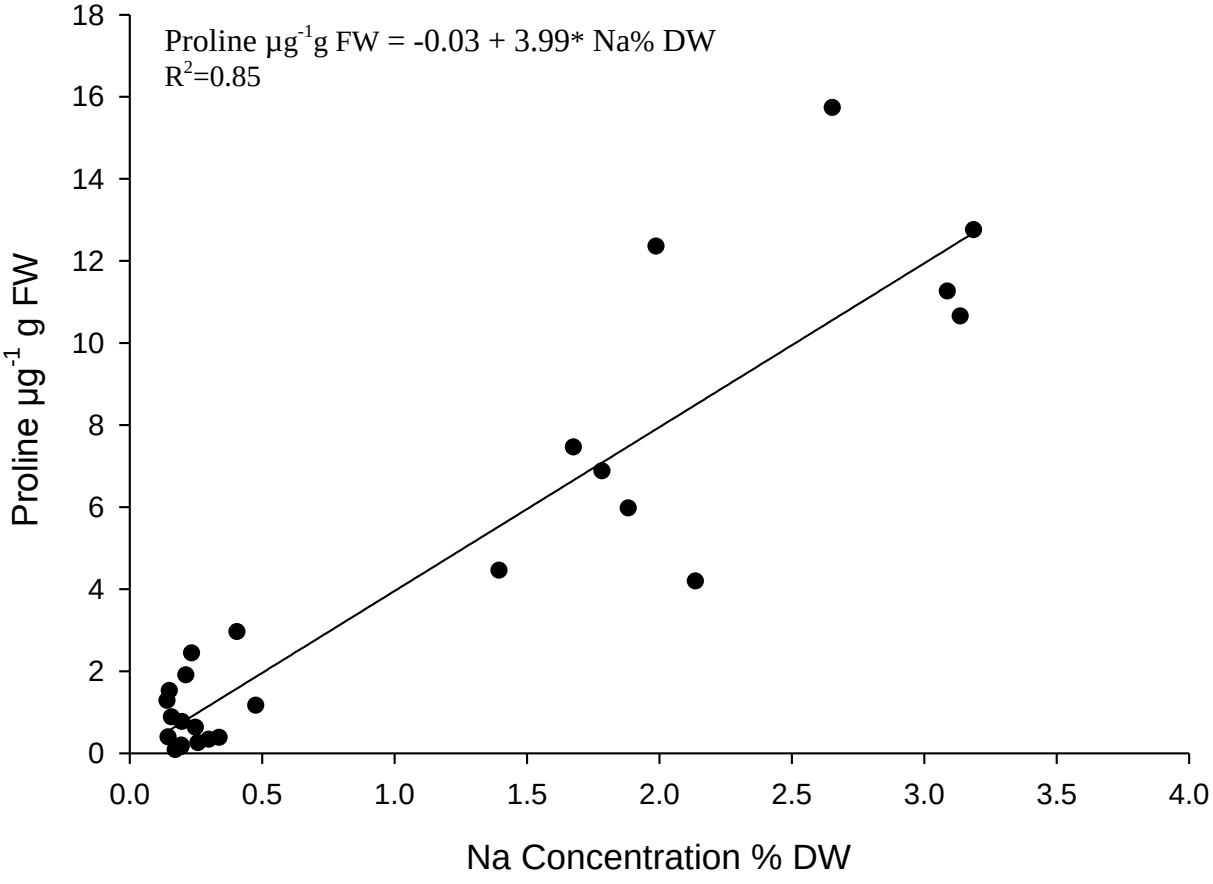


Figure 3.7. Regression of Na^+ concentration (%DW) and proline concentration (μg^{-1} g FW) in Champion at the conclusion of the study at the Clemson University Greenhouse Complex.

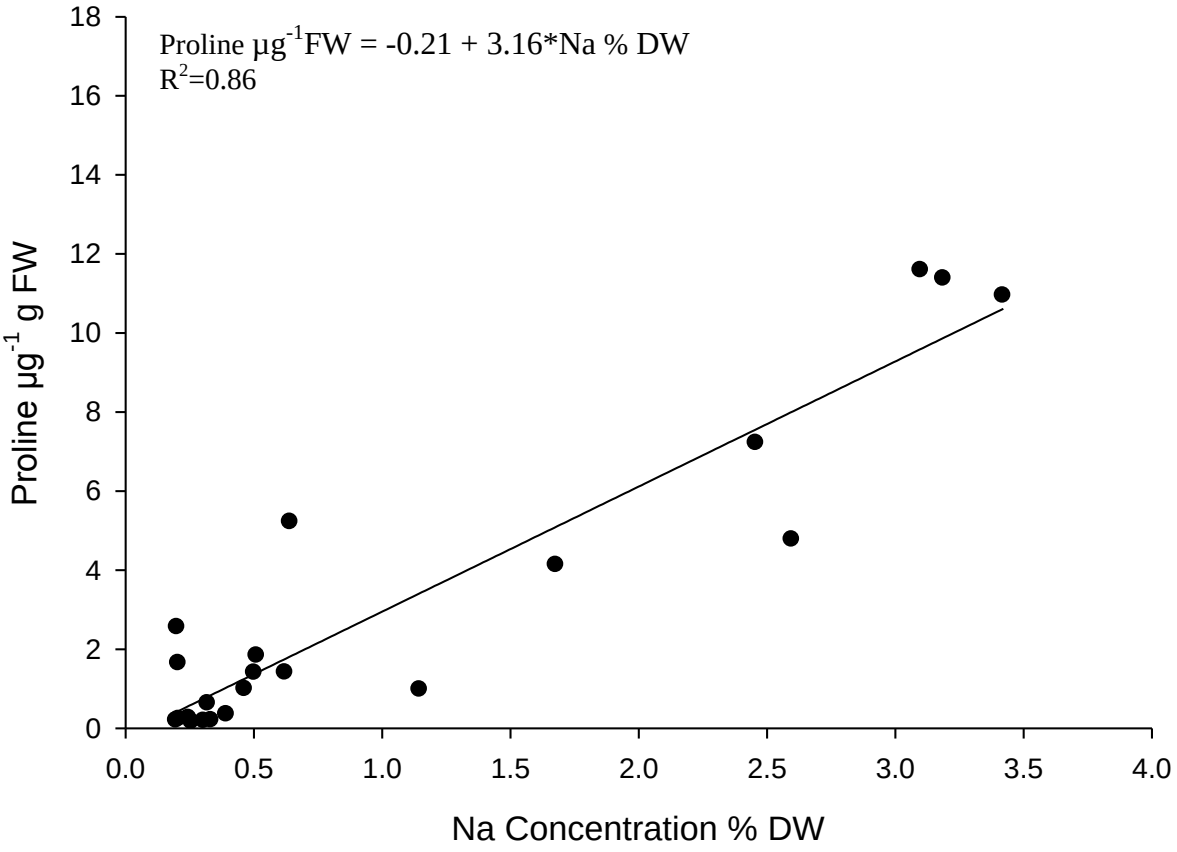


Figure 3.8. Regression of Na^+ concentration (%DW) and proline concentration (μg^{-1} g FW) in Diamond at the conclusion of the study at the Clemson University Greenhouse Complex.

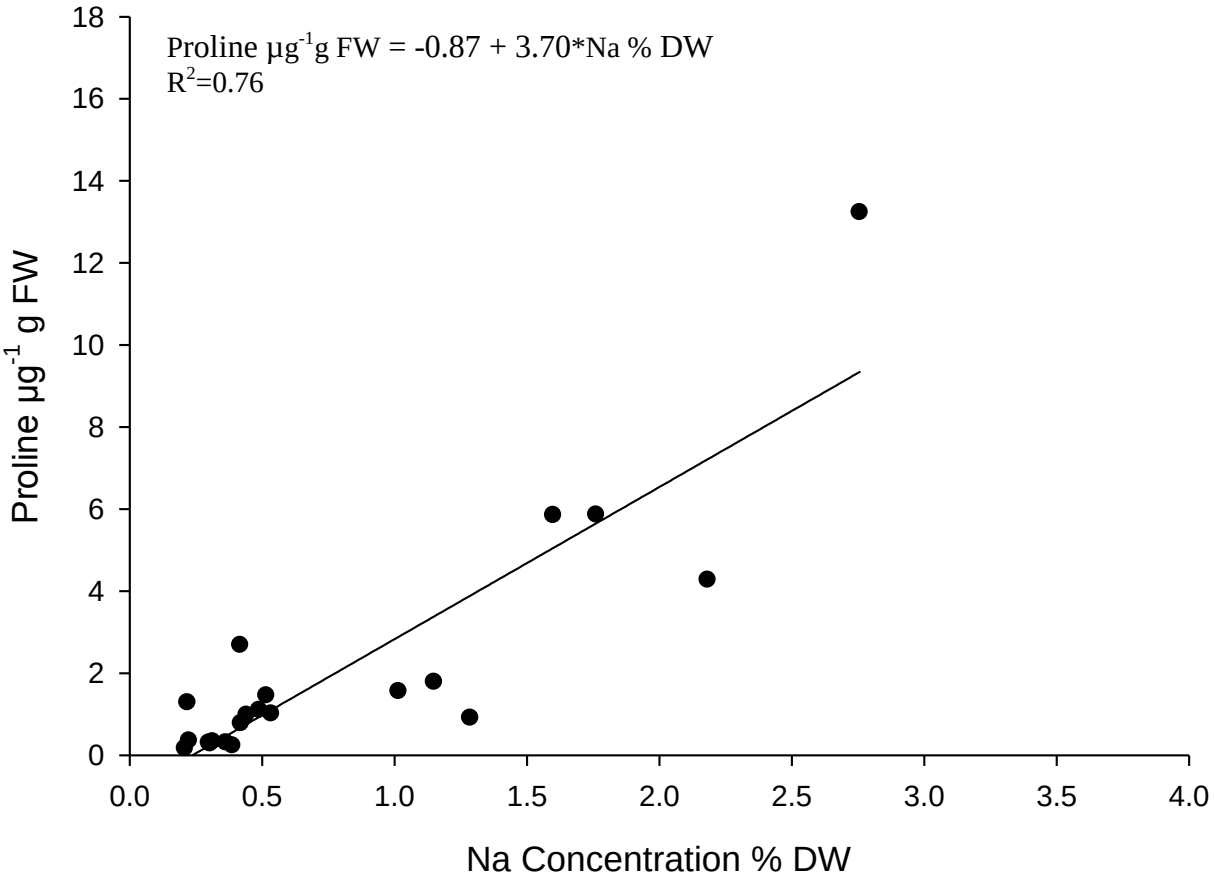


Figure 3.9. Regression of Na^+ concentration (%DW) and proline concentration (μg^{-1} g FW) in MiniVerde at the conclusion of the study at the Clemson University Greenhouse Complex.

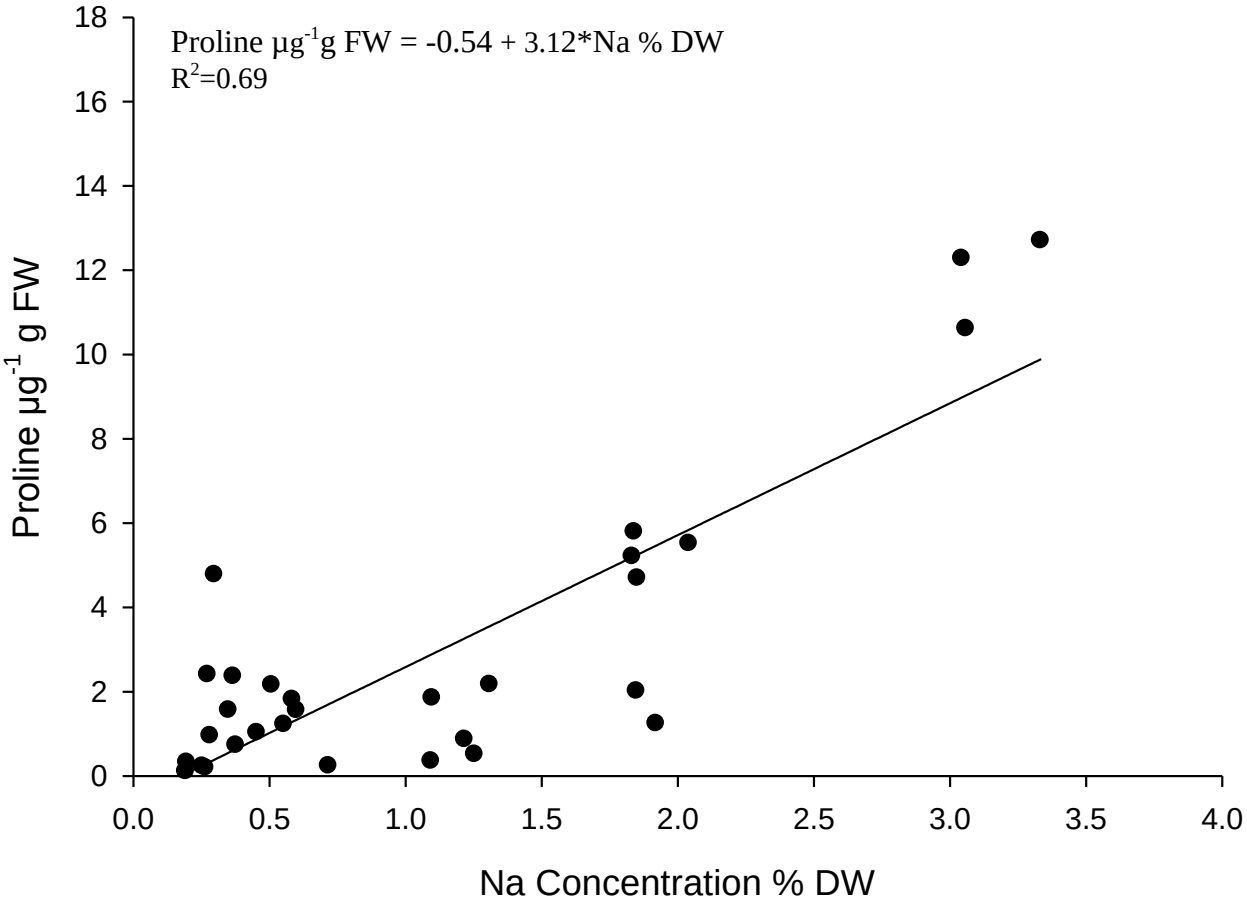


Figure 3.10. Regression of Na^+ concentration (%DW) and proline concentration (μg^{-1} g FW) in Seadwarf at the conclusion of the study at the Clemson University Greenhouse Complex.

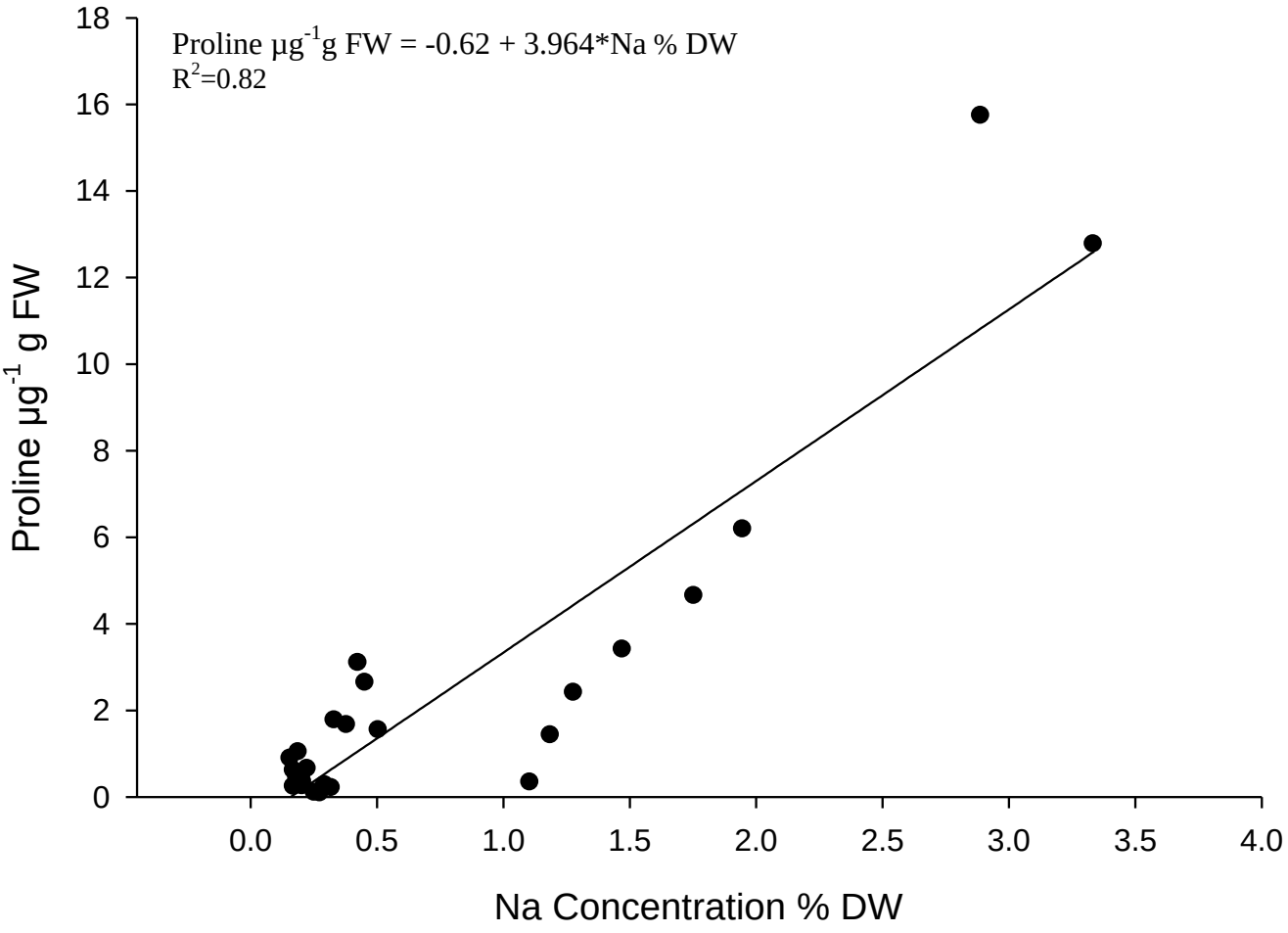


Figure 3.11. Regression of Na^+ concentration (%DW) and proline concentration (μg^{-1} g FW) in TifEagle at the conclusion of the study at the Clemson University Greenhouse Complex.

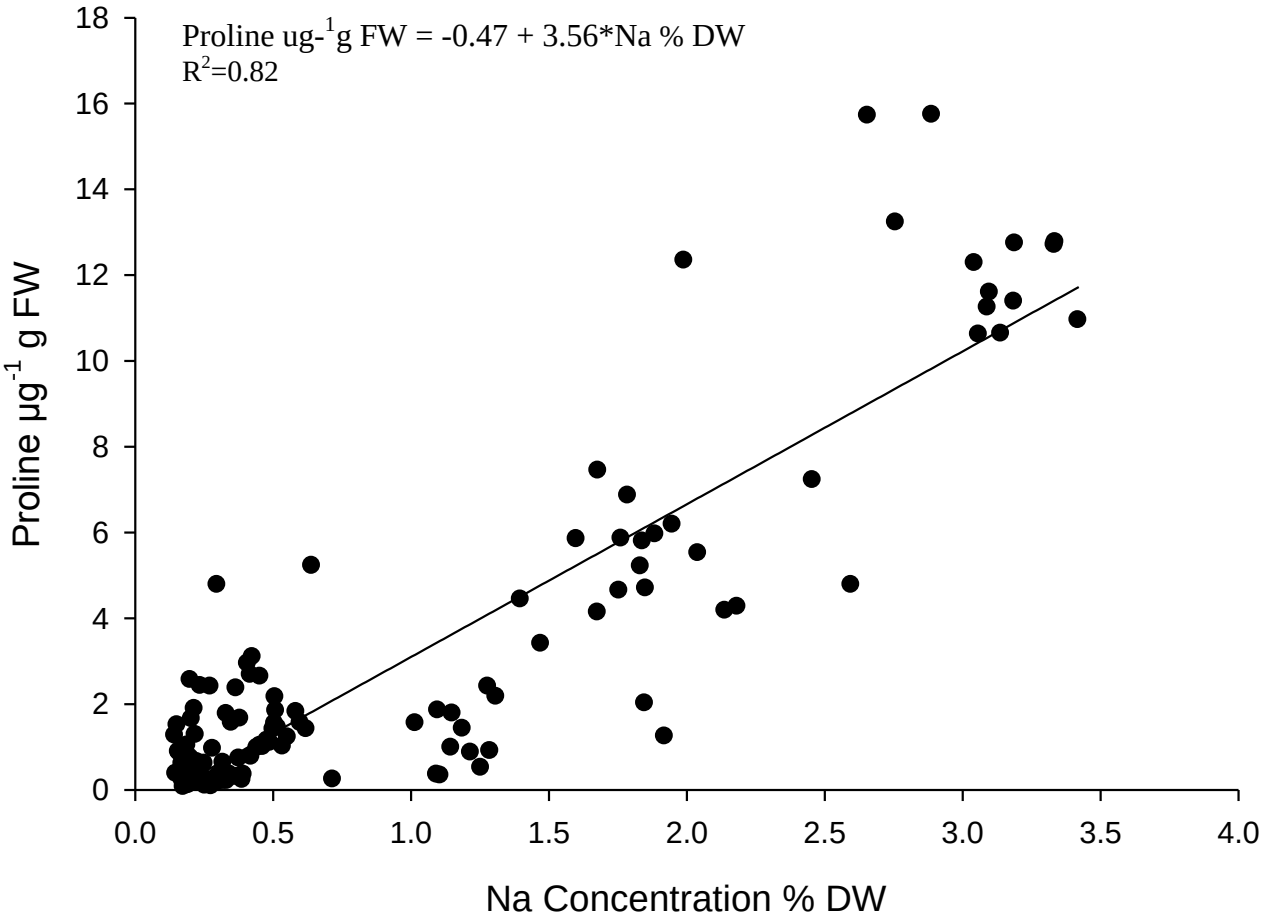


Figure 3.12. Regression of Na^+ concentration (%DW) and proline concentration (μg^{-1} g FW) in all genotypes at the conclusion of the study at the Clemson University Greenhouse Complex.

CHAPTER IV

SUPPLEMENTAL NICKEL APPLICATIONS AND FOLIAR UREA FERTILITY ON TWO WARM-SEASON TURFGRASS SPECIES UNDER SALINITY STRESS

Introduction

Due to the similarities displayed in root and foliar N uptake in the previous study, a second study was conducted to further examine foliar urea N fertility under salinity stress. Foliar fertilization is commonplace in turfgrass management and urea N is the most popular N source due to its solubility, high percentage of N by weight, low cost, and relatively low burn potential. However, before the N in urea is available to the plant to assimilate into organic N containing compounds it must be hydrolyzed by the Ni^{2+} containing enzyme urease. Stimulation of urea N metabolism has been observed in several plant species with Ni^{2+} supplementation (Yang et al., 1996; Gerendas and Sattelmacher, 1997b; Gerendas and Sattelmacher, 1999; Moraes et al., 2009). Due to the popularity of urea as a N source in turfgrass management, it is surprising that Ni^{2+} supplementation has not yet been researched to potentially improve urea N metabolism through the stimulation of urease. This is the first study examining Ni^{2+} supplementation and urea N metabolism of several warm-season turfgrasses under salinity stress. TifEagle bermudagrass and Diamond zoysiagrass were chosen due their salt tolerance, and increasing popularity in turfgrass management. This study was conducted to further examine their response to Ni^{2+} supplementation under foliar applied urea N and moderate salinity stress.

This study was designed to 1) assess urease activity in leaf tissue of two turfgrass species after foliar applications of urea N, 2) determine the effect of supplemental Ni^{2+} applications on urease activity and amino acid content, 3) determine if moderate salinity reduces urease activity and N metabolism in warm-season turfgrass leaf tissue, and 4) establish if Ni^{2+} supplementation increases foliar urea N uptake and assimilation.

Materials and Methods

Experiments included two repeated studies at the Clemson University Greenhouse Research Complex. Study 1 was conducted from May-August 2011 Greenhouse conditions averaged 27.2°C temperature and 61.5 % relative humidity. Average maximum and minimum temperatures were 33.4°C and 20.83°C respectively. Study II was conducted from July-October 2011. Greenhouse conditions averaged 26.5°C temperature and 59.7% relative humidity. Average maximum and minimum temperatures were 32.9°C and 18.8°C respectively. Twenty four 15.24 cm plugs of each species TifEagle, ultradwarf bermudagrasses and Diamond zoysiagrass were harvested from the Clemson turfgrass research plots and thoroughly washed to remove soil before being established in hydroponic culture. Each plug was placed into a 15.24 x 40 cm capped PVC tube supplied with continuous aeration. Each plug was supported using 0.3175 cm wooden dowel rods. Air was supplied with aquarium air pumps (Aqua Culture MK-1504, Bentonville, AR) and 0.64 cm black flex PVC airline fit into 22.86 cm disposable Pasteur pipettes (Fisher Scientific) to ensure proper depth in the hydroponic solution allowing for consistent aeration. The plant material was established in nutrient solution derived from Hoagland and Arnon (1950) with all macro and micronutrients supplied

(Table 4.1). Salinity was induced in three stages during establishment, beginning with 2,000 ppm NaCl and 4,000 ppm NaCl the two weeks prior to treatment commencement. For the duration of the study, N was removed from the hydroponic solution and 5,000 ppm NaCl was maintained for salinity treatments (Table 4.1). During establishment and study the hydroponic solution was replaced weekly to maintain proper levels and nutrient concentrations. Treatments consisted of two salinity levels (0 and 5,000 ppm NaCl) and three Ni^{2+} levels supplied as $NiCl_2$ [Control, 200 µg Ni^{2+} L^{-1}, and 400 µg Ni^{2+} L^{-1} (Table 4.1.)] to determine the effects of salinity stress and supplemental Ni^{2+} applications on N metabolism. Weekly foliar applications of urea Nat 9.8 kg ha^{-1} at a carrier volume of 561 L^{-1} ha^{-1} were conducted to supply 0.81 kg N for the duration of the study. Applications of bifenthrin (Talstar GC flowable) at 1.04L ha^{-1} were made three times during the establishment for control of Banks grass mites (*Oligonychus pratensis).*

Turfgrass Harvest and Parameters Measured

Turfgrass clippings were harvested at three, six, and nine weeks of the study. Roots were harvested at the conclusion of the studies and kept at 80°C until further analysis. Parameters measured in turfgrass clippings included: urease activity, total amino acid content, and tissue nutrient concentration.

Urease Assay

Methodology is listed in appendix B.

Amino Acid Assay

Methodology is listed in appendix B.

Mineral Nutrient Concentration Analysis

Tissue nutrient concentrations were determined by the following methodologies: Tissue N analysis was done by combustion utilizing a LECO FP528 N combustion analyzer (St. Joseph, MI). Mineral analysis of leaf tissue for P, K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, Fe, and S by HNO_3/H_2O_2 digestion, then analysis with ICP mass spectrometry. Leaf tissue Na^+ concentration was determined by weighing 1.0 gram sample into a 150 mL beaker. One hundred milliliters H_20 was added and placed on stirrer for 30 min. The mixture was filtered with metal filter and pour filtrate into a large test tube. Analysis was conducted using ICP mass spectrometry.

Data Analysis

Data were analyzed by ANOVA with JMP 9.0 (SAS Institute Inc. Cary, NC). Mean separations were performed using a Fisher's protected LSD test at 5% probability level.

Results

Root mass

Root mass was significantly influenced by the main effect of irrigation regime and cultivar. Saline irrigation significantly increased root mass over fresh water treatments. Overall, the root mass of salinity stressed plants was 125.96 g m^{-2}, whereas fresh water treatments exhibited an overall root mass of 112.05 g m^{-2}**.** Diamond exhibited significantly higher root mass than TifEagle. At the conclusion of the study, the overall root mass of Diamond was 169.10 g m^{-2} and only 68.92 g $m^{-2}$for TifEagle. Overall, the mean root mass of the second run was higher than the first run at 126.24 and 111.78 g m^{-2}. No significant interactions took place.

Clipping Yield

Clipping yield was determined at the conclusion of the study. All green leaf tissue was excised from each turfgrass sample, completely dried and weighed. All three main effects significantly influenced clipping yield at the conclusion of the study. Salinity treatments resulted in an average clipping yield of 490 g m^{-2} compared to 449.04 g m^{-2} for fresh water treatments (Figure 4.7). Nickel supplementation increased dry matter production in both turfgrass species examined. Clipping yields of 499.98, 466.80, and 442.01 g m^{-2} were exhibited for 400 μg L^{-1}, 200 μg L^{-1} and control Ni^{2+} treatments respectively. Diamond exhibited significantly higher clipping yield than TifEagle and the conclusion of the study (Figure 4.8). Average clipping yield for Diamond was 550.96 g m^{-2} compared to 388.23 g m^{-2} for TifEagle. A significant salinity*species interaction was exhibited. Diamond had similar clipping yields under each salinity level; however, TifEagle displayed an increase in clipping yield under salinity treatments. Under salinity treatments, TifEagle exhibited an average clipping yield of 426.80 g m^{-2} compared to only 349.67 g m^{-2} for fresh water treatments. Turfgrass growth can be visualized in Figure 4.1.

N Concentration

N concentration in leaf tissue (% DW) was determined at 3 harvests (three, six and nine weeks) during the study. At three weeks after the initiation of treatments species significantly influenced N concentration in leaf tissue (Table 4.2). TifEagle exhibited significantly higher N concentration in leaf tissue at 3.33% compared to Diamond at 3.02%. The overall effect of run was significant in the N concentration at

three weeks. Run 2 exhibited significantly higher N concentration in leaf tissue at 3.53% compared to 2.81% for run 1. At six weeks, TifEagle displayed significantly higher N concentration at 2.87% N in leaf tissue while Diamond exhibited 2.59 %. In addition to a species main effect, Ni^{2+} level significantly influenced N concentration. Control plots receiving no supplemental Ni^{2+} applications exhibited the greatest N concentration at 2.90% while 200 and 400 μg Ni^{2+} treatments exhibited similar N concentrations at 2.64 and 2.65 % respectively. This is the only harvest date where a reduction in N content in leaf tissue was caused by supplemental Ni^{2+} applications (Table 4.2). The overall effect of run was significant in the N concentration at six weeks. Run 2 exhibited significantly higher N concentration in leaf tissue at 3.09% compared to 2.37% for run 1. At the conclusion of the run the only main effect that significantly influenced N concentration in leaf tissue was run. Run 1 exhibited overall N concentrations of 1.77 % DW whereas run 2 exhibited 2.15 % DW.

Micronutrient Concentration

Reductions in manganese (Mn^{2+}) concentration were exhibited under Ni^{2+} supplementation. At three weeks, Mn^{2+} concentrations were 177.54, 164.20, and 156.38 mg kg^{-1} for 0, 200 and 400 μg Ni^{2+} L^{-1} treatments respectively. Six weeks after treatment initiation, Mn^{2+} concentrations in leaf tissue showed reductions at the highest Ni^{2+} supplementation level. Mn^{2+} concentrations were 214.52 and 211.28 mg kg^{-1} for 200 μg Ni^{2+} L^{-1} and control treatments whereas Mn^{2+} levels in leaf tissue in 400 μg Ni^{2+} L^{-1} only exhibited 190.23 mg kg^{-1}. This same trend was observed at the conclusion of the study. Mn^{2+} concentrations were 278.59 and 273.66 mg kg^{-1} for control and 200 μg Ni^{2+} L^{-1}

treatments whereas Mn^{2+} levels in leaf tissue in 400 μg Ni^{2+} L^{-1} only exhibited 253.53 mg kg^{-1}.

Magnesium (Mg^{2+}) concentration in leaf tissue was also reduced by Ni^{2+} supplementation at three and six weeks. Supplemental Ni^{2+} levels of 200 and 400 μg Ni^{2+} L^{-1} exhibited significantly lower Mg^{2+} concentrations at 1700.02 and 1670.90 mg kg^{-1} compared to the control at 18330 mg kg^{-1}. At six weeks, control and 200 μg Ni^{2+} L^{-1} treatments exhibited similar Mg^{2+} concentrations in leaf tissue. Plants receiving 400 μg Ni^{2+} L^{-1} displayed significantly lower Mg^{2+} concentrations at 1506.96 mg kg^{-1}than control at 1625.13 mg kg^{-1}.

Calcium concentration was reduced by Ni^{2+} supplementation at six weeks of the study. Only the highest Ni^{2+} level (400 μg Ni^{2+} L^{-1}) reduced Ca^{2+} levels compared to control and 200 μg Ni^{2+} L^{-1} treatments. Other micronutrients including iron, zinc and copper were not significantly influenced by Ni^{2+} supplementation.

Nickel Concentration

At three weeks after the initiation of treatments, Ni^{2+} level, species, and run main effects significantly influenced Ni^{2+} concentration in leaf tissue (Table 4.3). As the Ni^{2+} level increased so did the Ni^{2+} concentration in leaf tissue. Concentrations of 1.75, 1.12, and 0.42 mg kg^{-1} were exhibited for 400, 200 and control Ni^{2+} levels (Figure 4.6). At three weeks, Diamond exhibited significantly higher Ni^{2+} concentrations at 1.30 mg kg^{-1} in leaf tissue compared to TifEagle which displayed >1 mg kg^{-1} Ni^{2+} concentration. Overall Ni^{2+} levels were greater in run 1 at 1.21 mg kg^{-1} compared to run 2 where Ni^{2+} concentration in leaf tissue was 0.99 mg kg^{-1}. A significant Ni^{2+} level*species interaction

took place at all harvest dates (Table 4.6). At six weeks, all three main effects significantly influenced Ni^{2+} concentration in leaf tissue. Irrigation regime significantly influenced Ni^{2+} concentration in leaf tissue with salinity irrigation resulting in higher concentrations than fresh water irrigation. Ni^{2+} concentrations of 1.48 and 1.13 mg kg^{-1} were exhibited for salinity and fresh water irrigation respectively. The Ni^{2+} level significantly affected Ni^{2+} concentration in leaf tissue at six weeks. As the Ni^{2+} level increased so did the concentration in leaf tissue. Concentrations of 2.30, 1.31, and 0.31 mg kg^{-1} were exhibited for 400, 200 and control Ni^{2+} levels. At six weeks, Diamond exhibited significantly higher Ni^{2+} concentrations at 1.73 mg kg^{-1} in leaf tissue compared to TifEagle which displayed >1 mg kg^{-1} Ni^{2+} concentration. At nine weeks, all three main effects significantly influenced Ni^{2+} concentration in leaf tissue. Irrigation regime significantly influenced Ni^{2+} concentration in leaf tissue with fresh water irrigation resulting in higher concentrations than saline water irrigation. Nickel concentrations of 4.87 and 3.84 mg kg^{-1} were exhibited for fresh and saline water irrigation respectively. The Ni^{2+} level significantly affected Ni^{2+} concentration in leaf tissue at nine weeks. As the Ni^{2+} level increased so did the concentration in leaf tissue. Concentrations of 8.47, 4.25, and 0.35 mg kg^{-1} were exhibited for 400, 200 and control Ni^{2+} levels. At nine weeks, Diamond exhibited significantly higher Ni^{2+} concentrations at 6.18 mg kg^{-1} in leaf tissue compared to TifEagle which displayed 2.53 mg kg^{-1} Ni^{2+} concentration. At nine weeks, the Ni^{2+} concentration in leaf tissue was significantly higher in run 2 at 6.01 mg kg^{-1} compared to run 1 at 2.71 mg kg^{-1}.

Urease Activity

Urease activity was determined at three harvest dates (three, six, and nine weeks after the initiation of treatments) during each study (Table 4.4). Three weeks after the initiation of treatments, the urease activity was significantly affected by the main effects of Ni^{2+} level and species. Both 200 and 400 μg Ni^{2+} treatments resulted in increased urease activity compared to control treatments receiving no additional Ni^{2+}. Urease activity (expressed as μmol NH_4^+ min^{-1} g^{-1}) was 654.69, 563.60, and 185.13 for 400, 200, and 0 μg Ni^{2+} treatments respectively. Both supplemental Ni^{2+} treatments were not significantly different from each other. Diamond exhibited significantly higher urease activity than TifEagle at three weeks. Urease activities of 545.65 and 389.96 μmol NH_4^+ min^{-1} g^{-1} were displayed for Diamond and TifEagle correspondingly. At six weeks the only main effect to significantly influence urease activity was Ni^{2+} level. The same trend was observed with both supplemental Ni^{2+} levels (200 and 400 μg) resulting in significantly higher urease activity than control treatments (Figure 4.2). Both supplemental Ni^{2+} treatments exhibited similar urease activities at 883.16 and 797.57 μmol NH_4^+ min^{-1} g^{-1}. Experimental units not receiving supplemental Ni^{2+} applications exhibited significantly lower urease activity at 160.46 μmol NH_4^+ min^{-1} g^{-1}. The same trend was exhibited at the conclusion of the study. Both supplemental Ni^{2+} application rates resulted in increased urease activity. Urease activities of 733.85 and 664.70 μmol NH_4^+ min^{-1} g^{-1}were exhibited for 400 and 200 ug Ni^{2+} respectively. Control treatments (0 μg Ni^{2+}) exhibited urease levels of 202.61 μmol NH_4^+ min^{-1} g^{-1}. At the conclusion of the first run, overall urease activity levels were higher than the second run. 626.50 and 441.30 μmol NH_4^+ min^{-1} g^{-1} FW urease levels were exhibited for the 1^{st} and 2^{nd} runs

respectively. Also, a significant salinity treatment* Ni^{2+} level interaction was exhibited. At the 200 μg Ni^{2+} level, experimental units under salinity stress exhibited significantly higher urease activity than fresh water irrigation. Urease activities of 757.46 and 571.93 μmol NH_4^+ min^{-1} g^{-1} were exhibited for salinity and fresh water irrigation treatments respectively.

Amino Acid Content

Amino acid content was determined at three harvest dates (three, six, and nine weeks after the initiation of treatments) during each study (Table 4.5). At three Weeks after the initiation of treatments, Ni^{2+} level, species, and run main effects were significant. Both supplemental Ni^{2+} applications increased the total amino acid content in leaf tissues significantly over control treatments (Figure 4.3). Amino acid contents of 19.19 and 17.99 mg g^{-1} FW were exhibited for 400 and 200 μg Ni^{2+} treatments respectively. Diamond had higher amino acid contents in leaf tissue than TifEagle at three Weeks. Amino acid contents of 18.22 and 15.50 mg g^{-1} FW were exhibited in Diamond and TifEagle respectively (Figure 4.4). Run 2 exhibited significantly higher amino acid contents at 19.04 mg g^{-1} FW than run 14.68 mg g^{-1} FW for run 1. At all harvests there was a significant Ni^{2+} level*species interaction (Table 4.7). Diamond exhibited significantly higher amino acid pools under 200 and 400 μg Ni^{2+} treatments than control treatments. These two supplemental Ni^{2+} levels resulted in amino acid pools greater in Diamond than TifEagle under the same treatment. There was a smaller range of amino acid contents in the three Ni^{2+} levels in TifEagle. Both 200 and 400 μg Ni^{2+} levels resulted in similar amino acid contents at 15.65 and 16.79 mg g^{-1} FW respectively,

while the control treatments exhibited 14.05 mg g^{-1}FW amino acid content, which was not significantly different from the 200 μg Ni^{2+} treatment.

At six weeks after treatment initiation, the main effects of Ni^{2+} level and species influenced amino acid content in leaf tissue of the turfgrasses. Supplemental Ni^{2+} applications increased total amino acid pools in leaf tissue at six Weeks. Both 200 and 400 μg Ni^{2+} treatments resulted in similar amino acid contents at 19.05 and 20.14 mg g^{-1} FW respectively. In addition to Ni^{2+} level, species had a significant influence on amino acid content with Diamond exhibiting 20.27 mg g^{-1} FW while TifEagle displayed 14.04 mg g^{-1} FW, a significant difference. At six weeks, there was a significant Ni^{2+} level*species interaction (Table 4.7). Diamond exhibited significantly higher amino acid pools under 200 and 400 μg Ni^{2+} treatments than control treatments. These two supplemental Ni^{2+} levels resulted in amino acid pools higher in Diamond than TifEagle under the same treatment. There was a smaller range of amino acid contents in the three Ni^{2+} levels in TifEagle. Both 200 and 400 μg Ni^{2+} levels resulted in similar amino acid contents at 15.12 and 14.70 mg g^{-1} FW respectively, while the control treatments exhibited 12.22 mg g^{-1} FW amino acid content, not significantly different from the 400 μg Ni^{2+} treatment.

At the conclusion of the study the main effects of Ni^{2+} level and species significantly influenced the amino acid content in leaf tissue. Both supplemental Ni^{2+} levels increased total amino acid content in leaf tissue above the control treatments not receiving additional Ni^{2+}. Amino acid contents of 48.36 and 48.11 mg g^{-1} FW were exhibited for 400 and 200 μg Ni^{2+} treatments respectively. Experimental units not

receiving supplemental Ni^{2+} applications displayed significantly lower total amino acid content at 26.55 mg g^{-1} FW at the conclusion of the study. Species also significantly influenced amino acid pools in leaf tissue, with Diamond exhibiting significantly higher levels than TifEagle. At the conclusion of the study amino acid levels of 44.68 and 37.35 mg g^{-1} FW were seen in Diamond and TifEagle respectively. The Ni^{2+} level*species interaction was significant at the conclusion of the study (Table 4.7). Diamond and TifEagle exhibited significantly higher amino acid pools under 200 and 400 μg Ni^{2+} regimes than control treatments. However, amino acid pools in Diamond were significantly higher than TifEagle under the same treatments. Under 200 μg Ni^{2+} applications, Diamond exhibited 55.33 mg g^{-1} FW while TifEagle had amino acid contents of 40.88 mg g^{-1} FW. The same trend was also seen in the 400 μg Ni^{2+} treatment also.

Discussion

The stimulating effect of Ni^{2+} supplementation on urea grown plants is well documented (Krogmeier et al., 1991; Gerendas et al., 1998; Gerendas and Sattelmacher, 1999; Tan et al., 2000). Supplemental applications of Ni^{2+} stimulated urease activity in the leaf tissue over the course of the study which agrees with the findings of Krogmeier et al. (1991), Gerendas et al. (1998), and Gerendas and Sattelmacher (1999) with soybean, rice and spring rape respectively. Supplemental Ni^{2+} in the nutrient solution also increased the total amino acid pool in leaf tissue, presumably due to enhanced urease activity, which agrees with Gerendas and Sattelmacher (1999). Gerendas and Sattelmacher (1999) contributed the increase in amino acid content to storage and

transport forms (Gln, Asn, Glu, and Asp), suggesting that Ni^{2+} supplemented plants posses a luxury N status, which leads to a high leaf N content, however, our findings were different. Although an apparent stimulation of N metabolism took place over the course of the study, overall N concentration in leaf tissue decreased.

Supplemental Ni^{2+} up to 0.1 mg^{-1} L^{-1} increased growth and chlorophyll content in urea fed canola and supplementation promoted growth of urea fed tomato reporting that enhancement in growth was probably due to improved urea N assimilation which also lead to increases in N content of leaf tissue (Tan et al., 2000; Bybordi and Gheibi, 2009), which did not occur in this study. Clipping yield (i.e. growth) increased by Ni^{2+} supplementation however; an overall reduction of N content was seen. An increase in dry matter production with Ni^{2+} supplementation might be the responsible for diluting the N content in leaf tissue, which disagrees with findings of Tan et al. (2000) which found increases in both growth and N content in leaf tissue of tomato.

Amino acid contents in leaf tissue were also significantly influenced by species. Over the course of the study, both Diamond and TifEagle accumulated amino acid contents in their leaf tissue, with Diamond exhibiting significantly more than TifEagle. However, TifEagle consistently displayed greater N concentrations in leaf tissue than Diamond over the course of the study; and both species experienced a reduction in N concentration as the study progressed. To further investigate this occurrence examination in specific amino acid pools and N metabolites needs to be conducted. Witte (2011) suggested that N starvation and decreases in growth could be the result of elevated amino acid contents and urea as a sole N source, which is a possibility in this study. Elevated

levels of total amino acids in leaf tissue might cause feedback inhibition and decrease the plant's need to absorb foliar applied urea N. This phenomenon could lead to excessive volatilization from the leaf surface and poor fertilizer efficiency following foliar applications of urea N.

An accumulation of Ni^{2+} in the leaf tissue was exhibited while N levels decreased which disagrees with findings of Tan et al. (2000) and Khoshgoftarmanesh et al. (2011) who found increases in N content in leaf tissue of lettuce with Ni^{2+} supplementation. Nickel accumulated throughout the course of the study; as the Ni^{2+} level increased in the nutrient solution, overall Ni^{2+} concentrations in leaf tissue increased respectively. This result agrees with findings of Tan et al. (2000) in tomato. Critical toxicity levels in plants are in the range of > 10 $\mu g\ g^{-1}$ dry weight for sensitive, and > 50 $\mu g\ g^{-1}$ dry weight in moderately tolerant species (Marschner, 1995). At 400 $\mu g\ Ni^{2+}\ L^{-1}$, the highest level of Ni^{2+} concentration in leaf tissue was 8.47 mg kg^{-1}. No visual toxicity symptoms were exhibited at this level in either species, suggesting that TifEagle and Diamond are not sensitive to elevated Ni^{2+} levels in leaf tissue. The control treatments, although exhibiting significantly reduced levels of urease activity and amino acid content did not express symptoms of N deficiency, therefore, it is likely that their Ni^{2+} concentrations in leaf tissue were well above critical Ni^{2+} deficiency levels. Findings of Gerendas and Sattelmacher (1999) showed that spring rape grown on urea with Ni^{2+} supplementation did not influence concentrations of N in leaf tissue; however, this was recorded at six weeks of the study.

Visual signs of Ni^{2+} toxicity were not recorded throughout the study, although interactions with micronutrients and N metabolism are possible. Reductions in Mn^{2+}, Mg^{2+}, and Ca^{2+} concentrations agree with Gerendas and Sattelmacher (1999) where concentrations of micronutrients were commonly found to be lower in the leaf tissue of spring rape with Ni^{2+} additions, however many micronutrients including Cu^{2+}, Zn^{2+} and Fe were not significantly influenced. Increasing Ni^{2+} supplementation levels could further exacerbate micronutrient deficiencies leading to reduced enzyme function and alteration of key physiological processes.

Salinity treatments did not significantly influence urease activity, amino acid content, or N concentration throughout the study. However salinity significantly altered concentrations of several nutrients including Ni^{2+} in leaf tissue. Salinity treatments increased Ni^{2+} concentration in leaf tissue at six weeks; however, the opposite was true at the conclusion of the study with fresh water treatments resulting in higher Ni^{2+} concentrations in leaf tissue. These findings suggest that moderate salinity stress can influence micronutrient uptake in warm season turfgrass species. In addition, this finding provides further evidence that Na^{+} and Ni^{2+} uptake rely on similar uptake strategies although the trend was not consistent throughout the duration of the study. Lastly, an increase in clipping yield and root growth was also exhibited with moderate salinity stress, indicating potential stimulation of growth. This response further supports the possibility of warm-season turfgrasses requiring greater Na^{+} for normal growth and physiological processes. Species utilizing C_4 metabolism commonly require Na^{+} for regenerating phosphoenolpyruvate, the substrate for the first carboxulation in the C4

pathways (Taiz and Zeiger, 2010). Further examination of Na's role and sufficiency levels needs to be determined in warm-season turfgrass species.

Conclusions

The stimulation of N metabolism under foliar urea nutrition with Ni^{2+} supplementation has been achieved in two warm season turfgrass species. Although urease activity and amino acid pools were increased under Ni^{2+} supplementation, an overall decrease in N concentration in leaf tissue was observed over the course of the nine week study. This finding could be the result of utilizing urea as the sole N source which can cause reduced growth and symptoms of N starvation. To overcome these potential problems with utilizing urea as the sole source of N, multiple N fertility sources (NO_3^-, NH_4^+) should be utilized. Salinity stress commonly reduces the macro and micronutrient status within plants. However, a stimulation of growth was displayed under moderate salinity stress which also led to increased Ni^{2+} concentration in leaf tissue. This result supports the possibility of many warm-season turfgrasses possessing greater Na^+ requirements for optimum growth, especially in hydroponic conditions where as tested in this experiment.

Nickel deficiency is not commonly seen in turfgrass management; however, when urea is being utilized as a foliar N source, monitoring Ni^{2+} levels in soil and plant tissue Ni^{2+} might prove beneficial. Many positive effects of Ni^{2+} supplementation have been recorded. Nickel deficiency in pecan and other fruit trees is becoming more common and it is possible that many horticulture crops possess a "hidden hunger" for Ni^{2+}. In addition, Wood et al. (2012) has recorded the positive effects of Ni^{2+} supplementation on

disease management of fruit trees. Future research should examine the positive effects of Ni^{2+} supplementation on disease management of turfgrasses.

Further research needs to be conducted to examine supplemental Ni^{2+} applications on warm season turfgrass supplied with various N sources and rates. Analysis of urea and specific amino acid concentrations in plant tissues needs to be conducted. Through examination of specific amino acids, it will be possible to more fully understand the uptake, assimilation, and translocation of foliar applied urea N under the influence of Ni^{2+} supplementation. The significance of Ni^{2+} supply depends on N source and critical levels of Ni^{2+} in turfgrass tissue need to be determined in those scenarios. Lastly a comprehensive investigation at Ni^{2+} nutrition needs to be conducted to determine positive and negative effects of Ni^{2+} supplementation, including Ni^{2+} toxicity in turfgrasses.

Table 4.1. Stock solutions and concentrations for pre-culture†, minus N‡, Ni^{2+}§, and NaCl¶, solutions based on Hoagland and Arnon (1950)

Nutrient	Stock Solution Concentration	Experimental Concentration
A. Ammonium acid phosphate, $NH_4H_2PO_4$	1M	2 mM
B. Potassium nitrate, KNO_3	1M	3 mM
C. Calcium nitrate $CaNO_3$	1M	2 mM
D. Magnesium sulfate, $MgSO_4$ *7H_2O	1M	1 mM
E. Potassium sulfate, K_2SO_4	0.5 M	2.5 mM
F. Magnesium sulfate, $MgSO_4$	1M	2 mM
G. Calcium Phosphate Monobasic, CaH_2PO_4	0.05M	1 mM
	(g/L)	1ml/L
H. Micronutrient Stock	2.86	
Boric Acid, H_3BO_3	1.81	
Manganese chloride, $MnCl_2$* 4 H_20	0.22	
Zinc sulfate, $ZnSO_4$ * 7 H_2O	0.08	
Copper sulfate, $CuSO_4$ * 5 H_2O	0.02	
Molybdic acid, MoO_3*H_2O	21.0	1ml/L
I. Fe (Sequestrene)	250	
J. Sodium Chloride, NaCl	0.81	
K. Nickel Chloride, $NiCl_2$*6$H2_O$		

†**Pre-Culture Solution**: The nutrient solution was prepared using the following mL of stock solution per liter of final solution: 2ml of stock A; 3ml of stock B; 2ml of stock C; 1ml of stock D; 1 ml of stock H; 1 ml of stock I.

‡**Minus N Solution**: The nutrient solution was prepared using the following mL of stock solution per liter of final solution: 5ml of stock E; 2 ml of stock F; 20 ml of stock G; 1 ml of stock H; 1 ml of stock I.

§Ni Treatments: The nutrient solution was prepared using the following mL of stock solution per liter of final solution: 1 ml of stock K for 200 μg/L Ni treatments; 2 ml of stock K for 400 μg/L Ni treatments.

¶ NaCl Treatments: The nutrient solution was prepared using the following mL of stock solution per liter of final solution: 10 ml/L of stock J.

Table 4.2. N concentration in leaf tissue of 'Diamond' zoysiagrass and 'TifEagle' bermudagrass as influenced by salinity regime, Ni level, and species in Clemson University Greenhouse Research Complex during 2011 at three harvest events (3,6 and 9 weeks after initiation of treatments).

Main effects	3 Week	6 Week	9 Week
	----------% DW----------		
Salinity (S)			
Control	3.21	2.74	1.99
5,000 ppm	3.13	2.72	1.92
Ni Level (µg L^{-1})			
Control	3.21	2.90	2.01
200	3.14	2.64	1.99
400	3.16	2.65	1.88
$LSD_{0.05}$	NS	0.14	NS
Species (SP)			
Diamond	3.02	2.59	1.90
TifEagle	3.33	2.87	2.02
Run (R)			
1	2.81	2.37	1.77
2	3.53	3.09	2.15
	ANOVA		
Source of variation†			
S	NS	NS	NS
Ni	NS	***	NS
SP	***	***	NS
R	***	***	***
S*Ni	NS	NS	NS
S*SP	NS	NS	NS
Ni*SP	NS	NS	NS
S*Ni*SP	*	NS	NS
S*R	NS	NS	NS
Ni*R	NS	NS	NS
S*Ni*R	NS	NS	NS
SP*R	***	*	NS
S*SP*R	NS	NS	NS
Ni*SP*R	NS	NS	NS
S*Ni*SP*R	NS	NS	NS

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Main effect and interactions including Run (R) exhibited differences in magnitude, not changes in trend.

Table 4.3. Ni concentration in leaf tissue of 'Diamond' zoysiagrass and 'TifEagle' bermudagrass as influenced by salinity regime, Ni level, and species in Clemson University Greenhouse Research Complex during 2011 at three harvest events (3,6 and 9 weeks after initiation of treatments).

Main effects	3 Week	6 Week	9 Week
	----------mg kg^{-1} ----------		
Salinity (S)			
Control	1.02	1.13	4.87
5,000 ppm	1.18	1.48	3.84
Ni Level (µg L^{-1})			
Control	0.42	0.31	0.35
200	1.12	1.31	4.25
400	1.75	2.30	8.47
$LSD_{0.05}$	0.25	0.28	1.09
Species (SP)			
Diamond	1.30	1.73	6.18
TifEagle	0.89	0.89	2.53
Run (R)			
1	1.21	1.28	2.71
2	0.99	1.33	6.01
ANOVA			
Source of variation†			
S	NS	**	*
Ni	***	***	***
SP	***	***	***
R	*	NS	***
S*Ni	NS	NS	NS
S*SP	NS	NS	NS
Ni*SP	***	***	***
S*Ni*SP	NS	NS	NS
S*R	**	NS	**
Ni*R	NS	NS	***
S*Ni*R	NS	NS	NS
SP*R	NS	*	*
S*SP*R	NS	NS	NS
Ni*SP*R	NS	NS	NS
S*Ni*SP*R	NS	NS	NS

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Main effect and interactions including Run (R) exhibited differences in magnitude, not changes in trend.

Table 4.4. Urease activity in leaf tissue of 'Diamond' zoysiagrass and 'TifEagle' bermudagrass as influenced by salinity regime, Ni level, and species in Clemson University Greenhouse Research Complex during 2011 at three harvest events (3,6 and 9 weeks after initiation of treatments).

Main effects	3 Week	6 Week	9 Week
	---------µmol NH_4^+ min^{-1} g^{-1}--------		
Salinity (S)			
Control	486.91	587.75	533.14
5,000 ppm	448.70	639.71	534.30
Ni Level (µg L^{-1})			
Control	185.13	160.46	202.61
200	563.60	797.57	664.70
400	654.69	883.16	733.85
$LSD_{0.05}$	134.50	170.91	134.80
Species (SP)			
Diamond	545.65	639.36	545.11
TifEagle	389.96	588.10	522.32
Run (R)			
1	486.61	587.81	626.13
2	449.01	639.65	441.30
	ANOVA		
Source of variation†			
S	NS	NS	NS
Ni	***	***	***
SP	**	NS	NS
R	NS	NS	**
S*Ni	NS	NS	*
S*SP	NS	NS	NS
Ni*SP	NS	NS	NS
S*Ni*SP	NS	NS	NS
S*R	NS	NS	NS
Ni*R	NS	NS	***
S*Ni*R	NS	NS	NS
SP*R	NS	NS	NS
S*SP*R	NS	NS	NS
Ni*SP*R	NS	NS	NS
S*Ni*SP*R	NS	NS	NS

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Main effect and interactions including Run (R) exhibited differences in magnitude, not changes in trend.

Table 4.5. Amino acid content in leaf tissue of 'Diamond' zoysiagrass and 'TifEagle' bermudagrass as influenced by salinity regime, Ni level, and species in Clemson University Greenhouse Research Complex during 2011 at three harvest events (3,6 and 9 weeks after initiation of treatments).

Main effects	3 Week	6 Week	9 Week
	----------mg g^{-1} FW----------		
Salinity (S)			
Control	16.73	16.61	42.43
5,000 ppm	16.99	17.69	39.59
Ni Level (µg L^{-1})			
Control	13.40	12.27	26.55
200	17.99	19.05	48.11
400	19.19	20.14	48.36
$LSD_{0.05}$	1.56	1.94	3.86
Species (SP)			
Diamond	18.22	20.26	44.66
TifEagle	15.50	14.04	37.35
Run (R)			
1	14.68	18.23	39.35
2	19.04	16.07	42.67
	ANOVA		
Source of variation†			
S	NS	NS	NS
Ni	***	***	***
SP	***	***	**
R	***	**	*
S*Ni	NS	NS	NS
S*SP	NS	NS	NS
Ni*SP	**	***	***
S*Ni*SP	NS	NS	NS
S*R	NS	NS	NS
Ni*R	NS	NS	NS
S*Ni*R	NS	NS	NS
SP*R	NS	NS	NS
S*SP*R	NS	NS	NS
Ni*SP*R	NS	NS	NS
S*Ni*SP*R	NS	NS	NS

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Main effect and interactions including Run (R) exhibited differences in magnitude, not changes in trend.

Table 4.6. Ni concentration (mg kg^{-1}) of leaf tissue of 'Diamond' zoysiagrass and 'TifEagle' bermudagrass as influenced by Ni level in Clemson University Greenhouse Research Complex during 2011 at3,6, & 9 weeks.

Species	Ni Level (µg L^{-1})	3 wk	6 wk	9 wk
		----------mg kg^{-1} ----------		
TifEagle			------	
	Control	0.47d	0.29d	0.36d
	200	0.96c	0.99c	2.75c
	400	1.44b	1.43b	6.10b
Diamond				
	Control	0.34d	0.30d	0.15d
	200	1.36b	1.64b	6.66b
	400	2.42a	3.26a	13.14a

Means within a column followed by the same letter are not significant different at $p \leq 0.05$ by protected LSD.

Table 4.7. Total amino acid content (mg^{-1} g^{-1}) of leaf tissue of 'Diamond' zoysiagrass and 'TifEagle' bermudagrass as influenced by Ni level in Clemson University Greenhouse Research Complex during 2011 at 3,6, & 9 weeks.

Species	Ni Level (µg L^{-1})	3 wk	6 wk	9 wk
		----------mg g^{-1}FW----------		
TifEagle			------	
	Control	14.05cd	12.32c	28.15c
	200	15.65bc	15.12b	40.88b
	400	16.79b	14.70bc	43.02b
Diamond				
	Control	12.74d	12.22c	24.96c
	200	20.33a	22.99a	55.33a
	400	21.6a	25.58a	53.70a

Means within a column followed by the same letter are not significantly different at $p \leq 0.05$ by protected LSD.

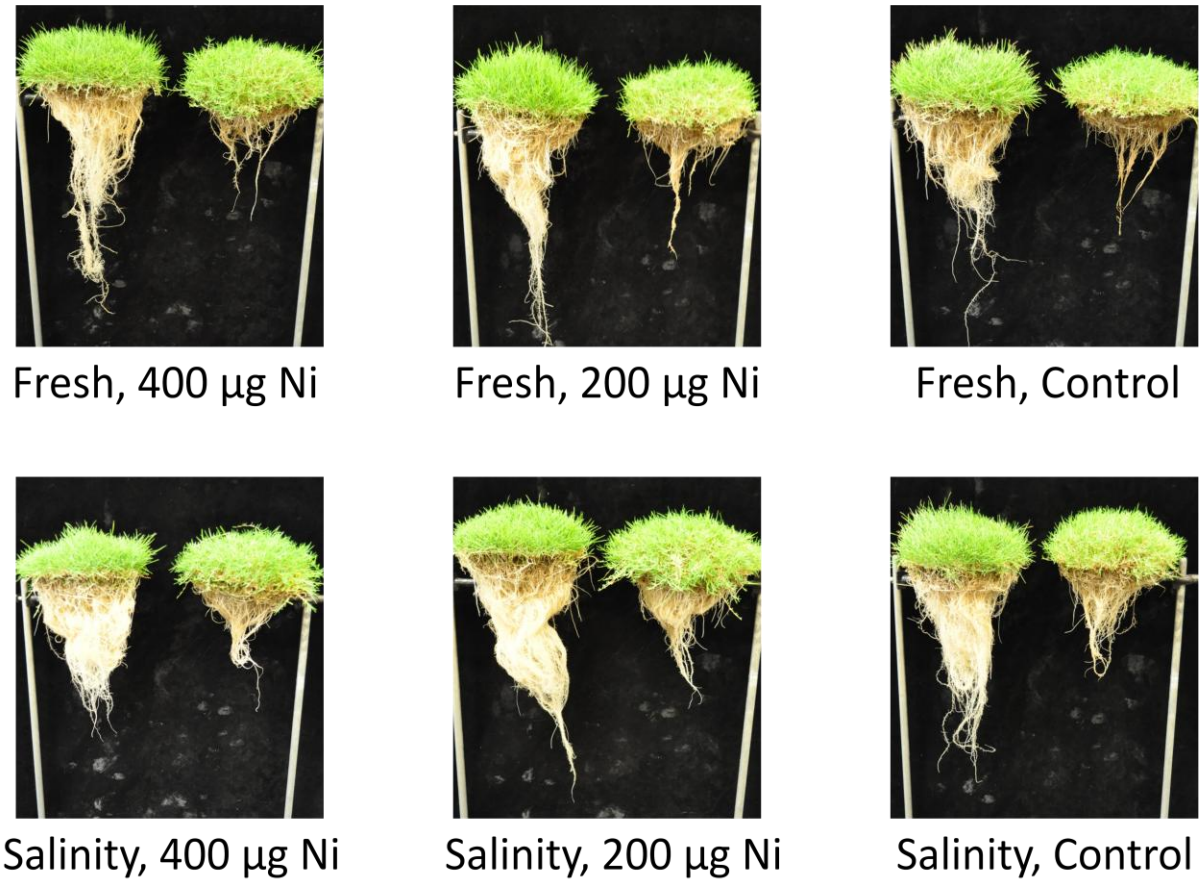


Figure 4.1. Images of Diamond (L) and TifEagle (R) growth under two salinity levels (Control, 5,000 ppm NaCl) and three Ni^{2+} supplementation regimes (Control, 200, and 400 µg L^{-1} Ni^{2+}) at the conclusion of the study.

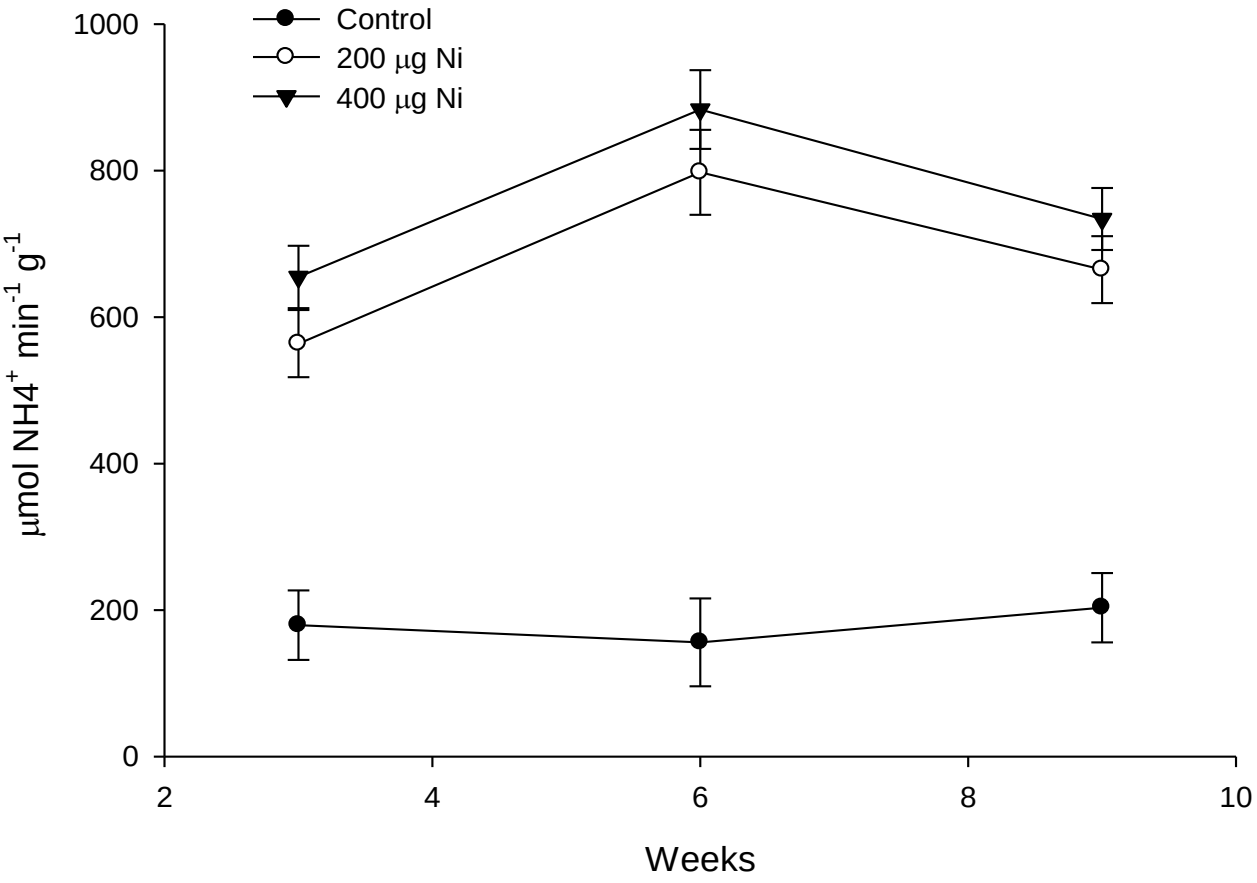


Figure 4.2. Urease activity (μmol NH_4^+ min^{-1} g^{-1}FW) in leaf tissue of 'Diamond'zoysiagrass and 'TifEagle' bermudagrass as influenced by Ni^{2+} level in the Clemson University greenhouse research complex over three harvest dates during 2011. Means were separated at $p \leq 0.05$ by protected LSD.

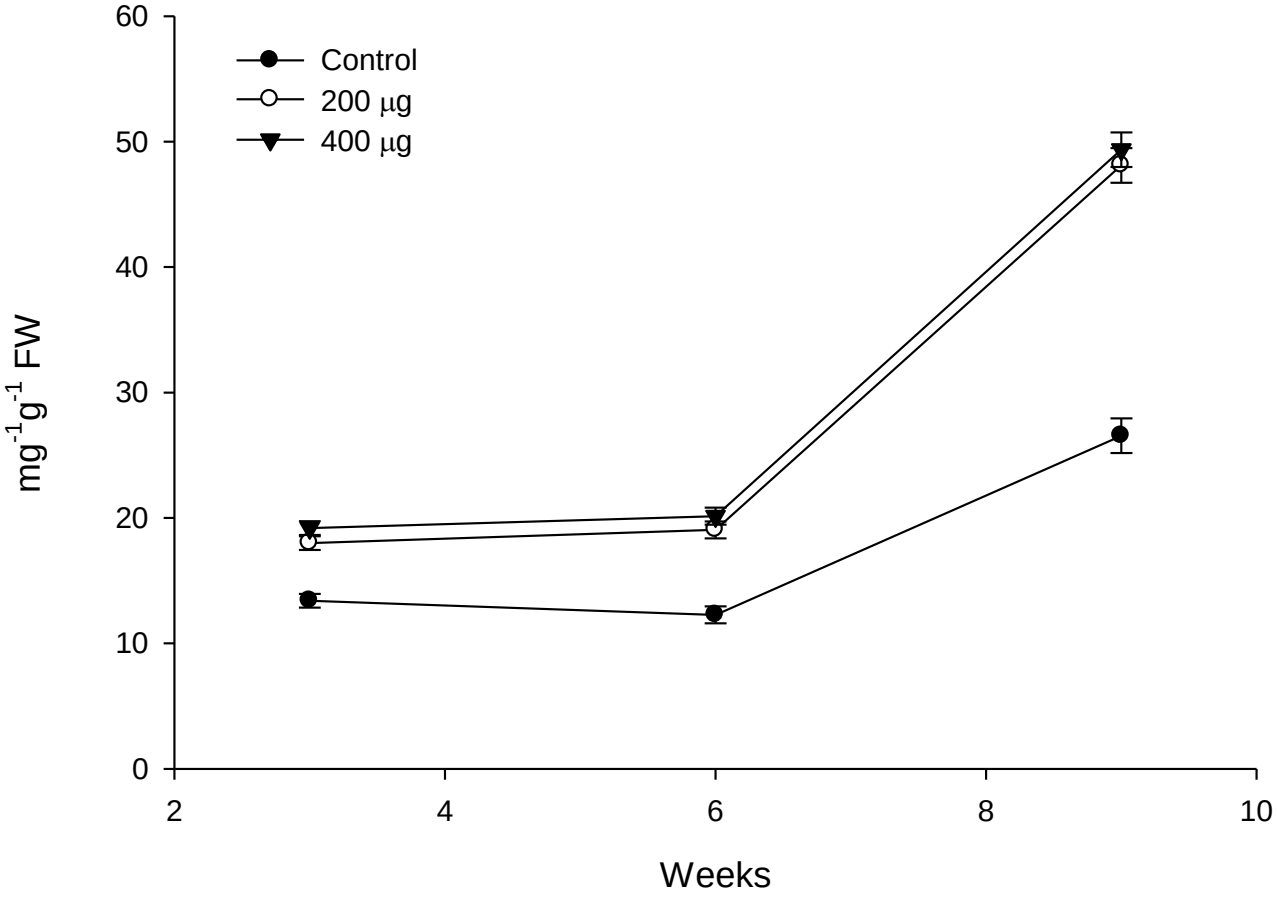


Figure 4.3. Total amino acid content (mg g^{-1} FW) in leaf tissue of 'Diamond' zoysiagrass and 'TifEagle' bermudagrass as influenced by Ni^{2+} level in the Clemson University greenhouse research complex over three harvest dates during 2011. Means were separated at $p \leq 0.05$ by protected LSD.

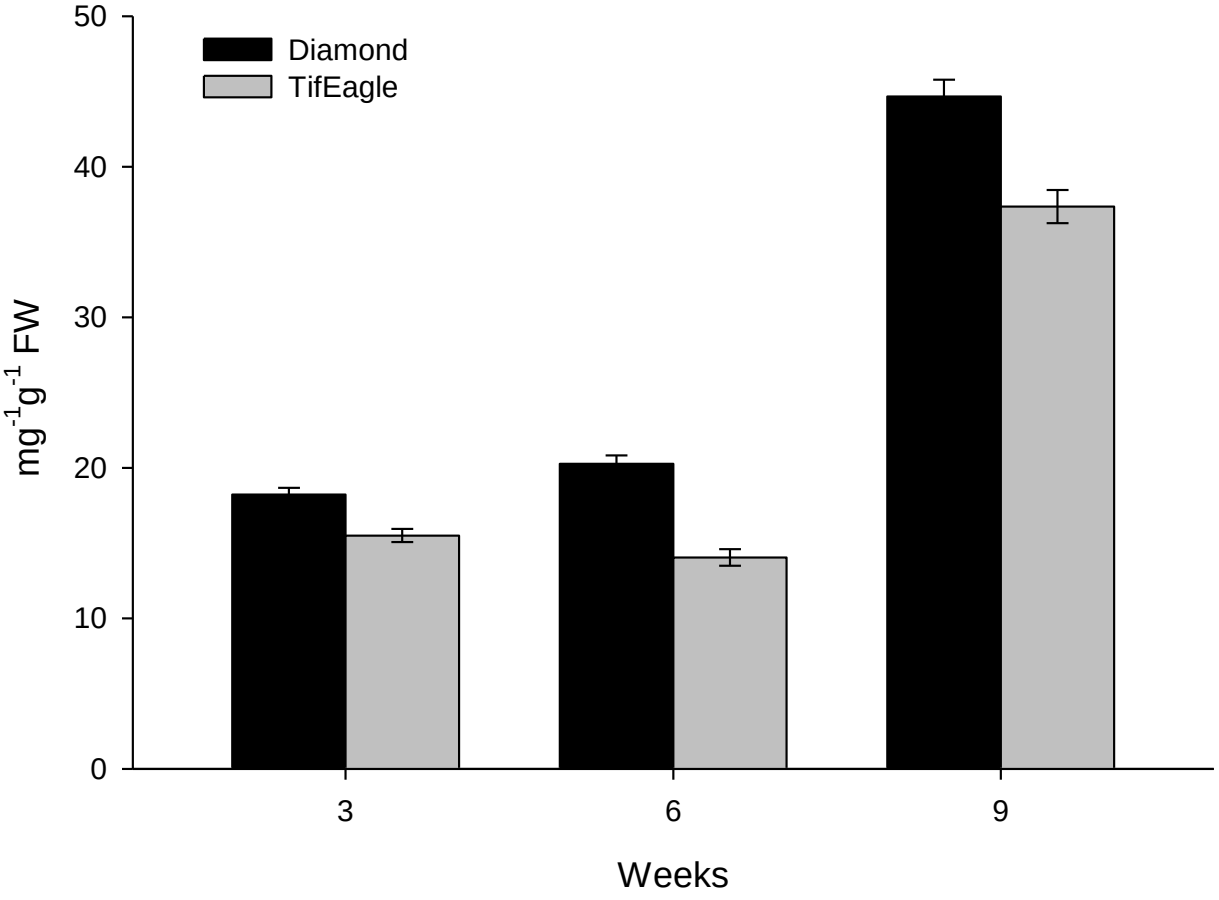


Figure 4.4. Total amino acid content (mg g^{-1}FW) in leaf tissue of 'Diamond' zoysiagrass and 'TifEagle' bermudagrass in the Clemson University greenhouse research complex over three harvest dates during 2011. Means were separated at $p \leq 0.05$ by protected LSD.

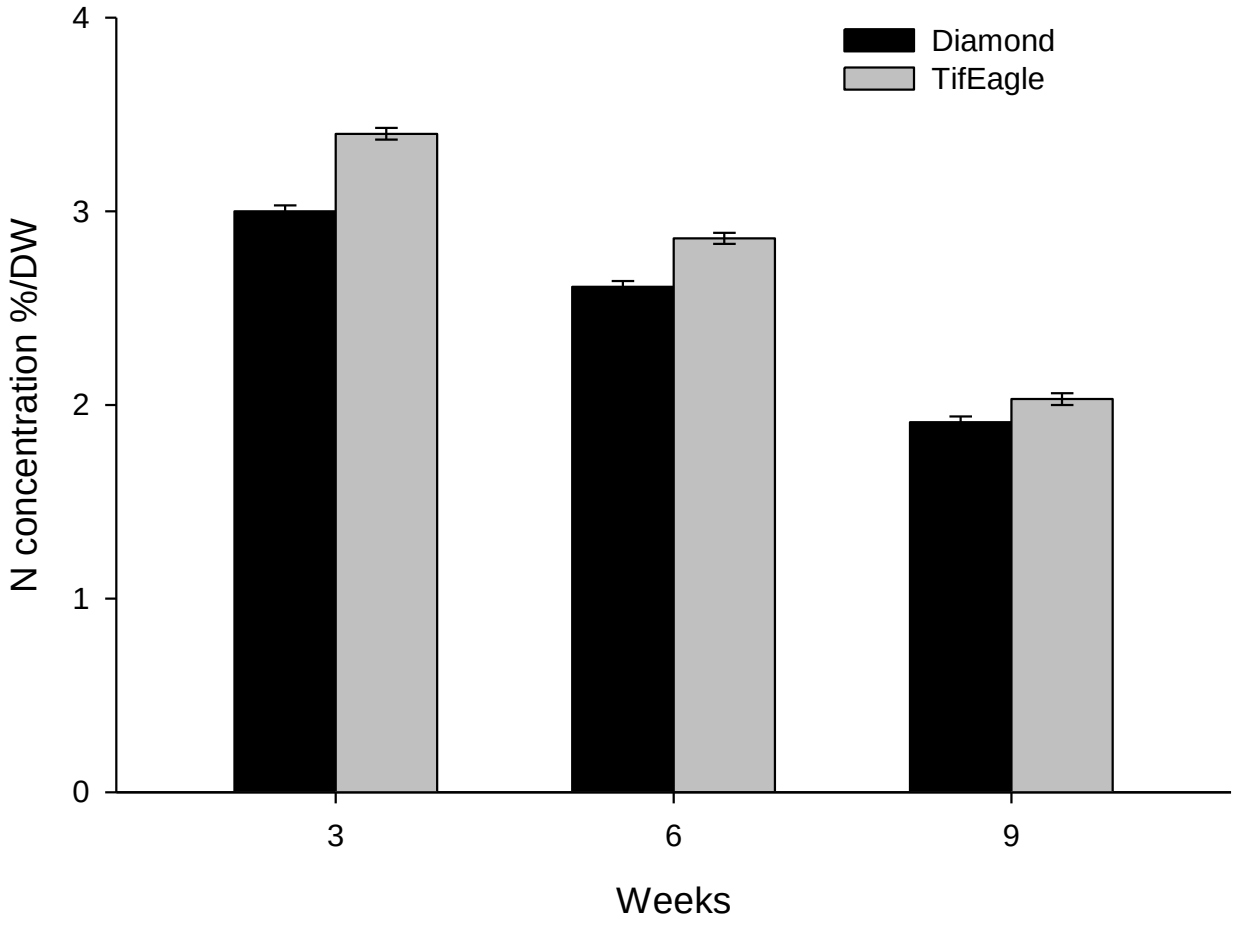


Figure 4.5. Nitrogen concentration (% DW) in leaf tissue of 'Diamond' zoysiagrass and 'TifEagle' bermudagrass in the Clemson University greenhouse research complex over three harvest dates during 2011. Means were separated at $p \leq 0.05$ by protected LSD.

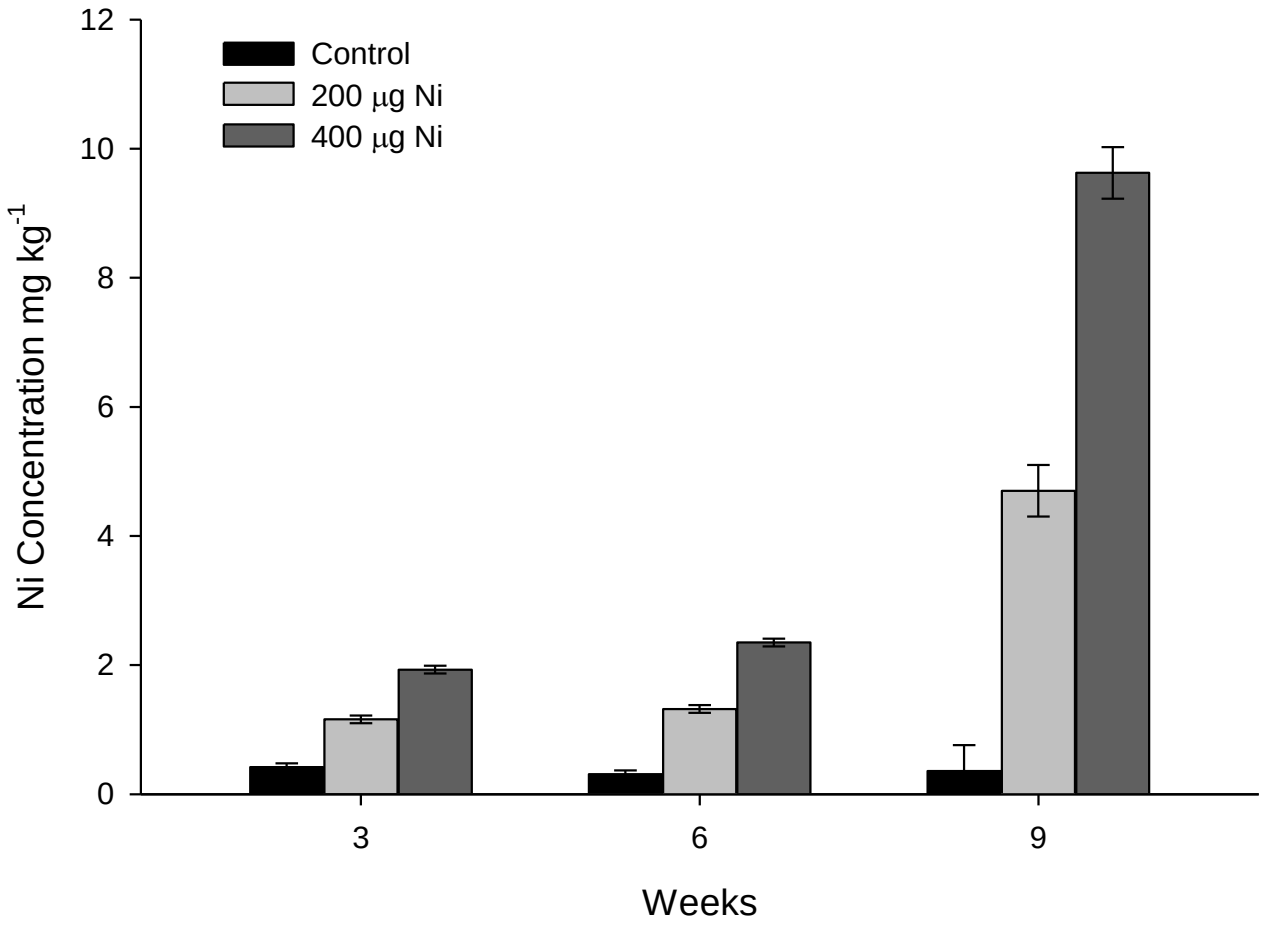


Figure 4.6. Nickel concentration (mg kg^{-1}) in leaf tissue of 'Diamond' zoysiagrass and 'TifEagle' bermudagrass in the Clemson University greenhouse research complex over three harvest dates during 2011. Means were separated at $p \leq 0.05$ by protected LSD.

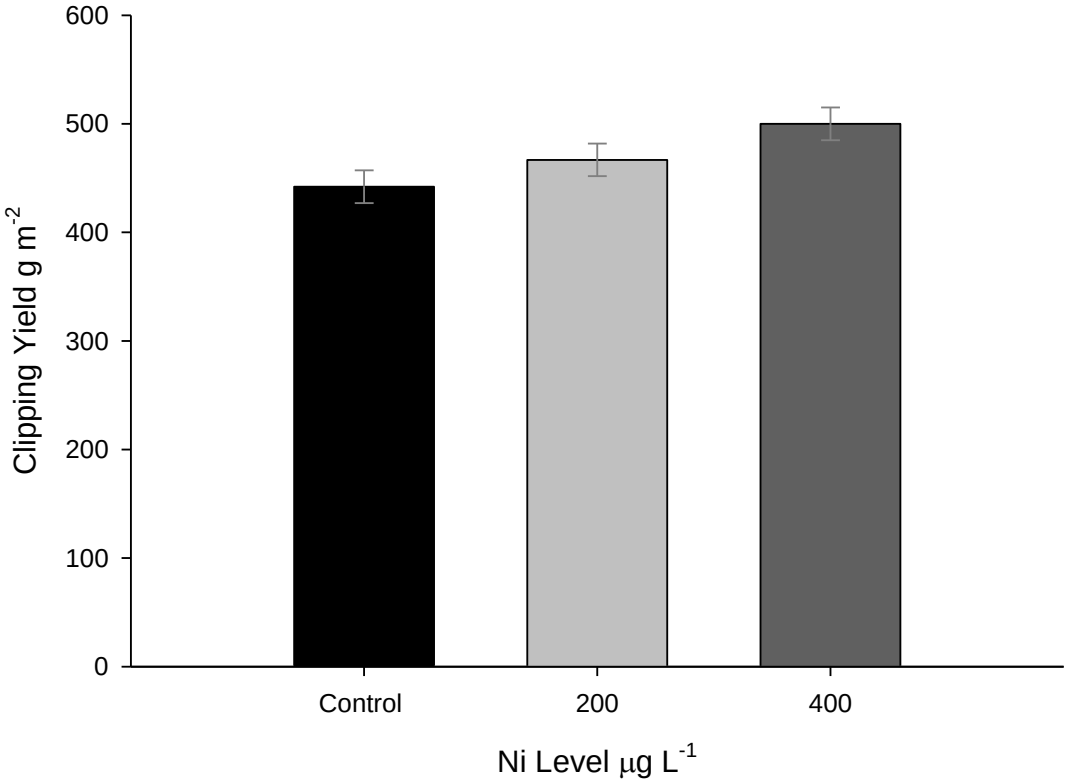


Figure 4.7. Main effect of Ni^{2+} level (μg L^{-1}) on clipping yield at the conclusion of the study in the Clemson University greenhouse research complex during 2011. Means were separated at $p \leq 0.05$ by protected LSD.

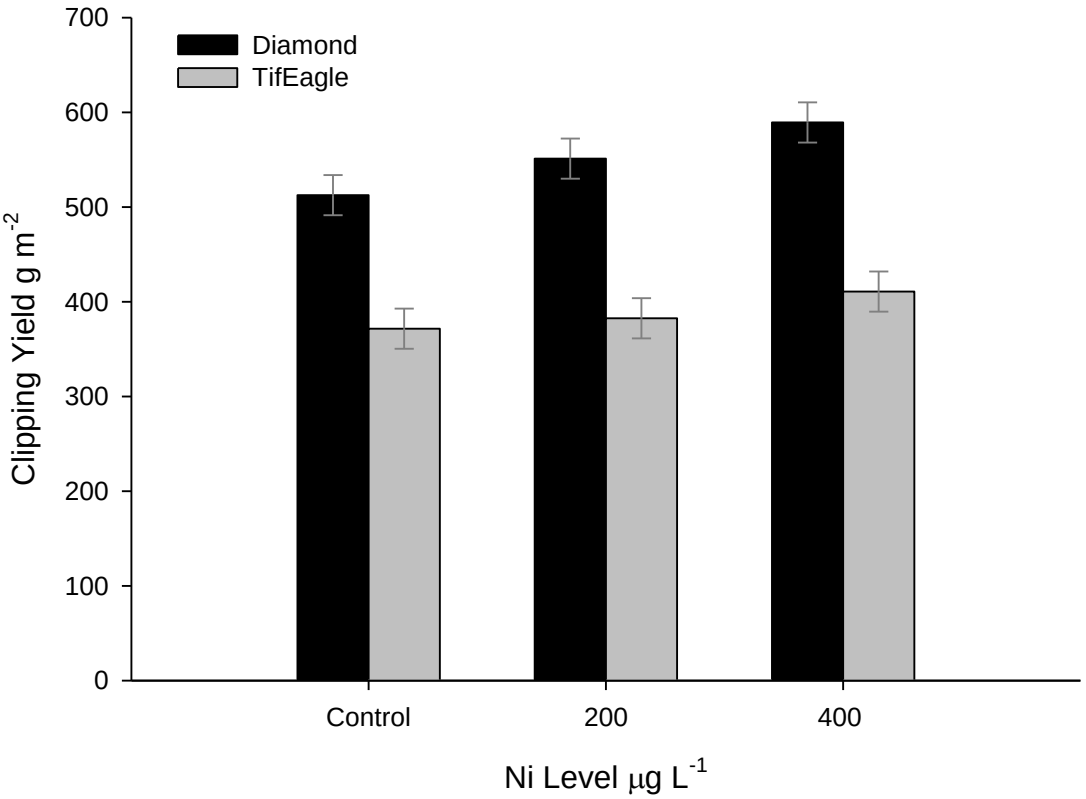


Figure 4.8. Clipping yield of Diamond zoysiagrass and TifEagle bermudagrass as influenced by Ni^{2+} level (μg L^{-1}) at the conclusion of the study in the Clemson University greenhouse research complex during 2011. Means were separated at $p \leq 0.05$ by protected LSD.

CHAPTER V

NICKEL TOXICITY AND UREA N METABOLISM IN TWO WARM-SEASON TURFGRASS SPECIES

Introduction

The stimulation of urea N metabolism by increases in urease activity and amino acid pools with Ni^{2+} supplementation in warm-season turfgrasses was achieved in the previous chapter. To further examine Ni^{2+} nutrition and urea N metabolism in TifEagle bermudagrass and Diamond zoysiagrass, a third study was conducted. Ni^{2+} deficiency is thought to not commonly occur in turfgrass management due to the very small concentration required for plant metabolism. Due to this fact there has been little research determining critical deficiency and toxicity levels in many plants, including turfgrasses.

Typically, an elevated level of Ni^{2+} in plant tissue is more common due to increased industrial pollution, and regions that possess serpentine soils containing elevated levels of Ni^{2+} (Reeves et al., 1999; Chen et al., 2009). Nickel toxicity can negatively affect plant health in a number of ways including: disruption of photosynthesis, induction of micronutrient deficiencies, oxidative stress, and reduction of growth. Currently there is a lack of research examining Ni^{2+} nutrition and toxicity in turfgrasses. Based upon the Ni^{2+} concentrations in the leaf tissue, overall health, and urea N metabolism of TifEagle and Diamond in the previous chapter, the goal of this study was to further examine the influence of Ni^{2+} on plant metabolism by significantly increasing Ni^{2+} supplementation levels.

This study was conducted to 1) assess urea N metabolism of two turfgrass species after foliar applications of urea nitrogen, 2)) determine critical Ni^{2+} toxicity levels in two warm-season turfgrasses, and 4) document Ni^{2+} toxicity symptoms and responses of two turfgrass species.

Materials and Methods

Experiments included two repeated studies at the Clemson University Greenhouse Research Complex. Study 1 was conducted from April 9th-May 21st 2012 (6 weeks). Greenhouse conditions averaged 25°C temperature and 59% relative humidity. Average maximum and minimum temperatures were 31°C and 18°C respectively. Study II was conducted from April 23rd-June 4th 2012 (6 weeks). Greenhouse conditions averaged 26.5°C temperature and 63% relative humidity. Average maximum and minimum temperatures were 31.5°C and 18.5°C respectively. Sixteen 15.24 cm plugs of each species: TifEagle', ultradwarf bermudagrasses and Diamond zoysiagrass were harvested from turfgrass research plots at Clemson University and transplanted into 15.24 cm pots and thoroughly watered and transferred into the Clemson University Greenhouse Research Complex. Evapotranspiration (ET) was determined gravimetrically over a 72 hour period to determine water loss/irrigation requirement over the course of the study. Prior to initiation of treatments, each pot was flushed with minus N nutrient solution derived from Hoagland and Arnon (1950) (Table 5.1). Nickel treatments were applied to replace ET 3x weekly. Weekly foliar applications of urea Nat 9.8 kg ha^{-1} at a carrier volume of 561 L^{-1} ha^{-1} were conducted with a CO_2 pressurized backpack sprayer. Treatments consisted of four Ni^{2+} levels supplied as $NiCl_2$ [Control, 400, 800, and 1600

μM (Table 5.1)] to determine the effects of supplemental Ni^{2+} applications on N metabolism. Weekly foliar applications of urea N at 9.8 kg ha^{-1} at a carrier volume of 561 L^{-1} ha^{-1} were conducted to supply 0.54 kg N for the duration of the study. Applications of lambda-cyhalothrin (Scimitar GC) at 538.2 ml ha^{-1} were made twice during the study for control of Banks grass mites (*Oligonychus pratensis).*

Turfgrass Harvest and Parameters Measured

Turfgrass clippings were harvested at the conclusion of the study. Roots were harvested at the conclusion of the studies and kept at 80°C until further analysis. Parameters measured in turfgrass clippings included: urease activity, total amino acid content, tissue nutrient concentration and clipping yield.

Urease Assay

Methodology is listed in appendix B.

Amino Acid Assay

Methodology is listed in appendix B.

Mineral Nutrient Concentration Analysis

Tissue nutrient concentrations were determined by the following methodologies: Tissue N analysis was done by combustion utilizing a LECO FP528 N combustion analyzer (St. Joseph, MI). Mineral analysis of leaf tissue for P, K^{+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, Fe, and S by HNO_3/H_2O_2 digestion, then analysis with ICP mass spectrometry. Leaf tissue Na^{+} concentration was determined by weighing 1.0 gram sample into a 150 mL beaker. One hundred milliliters H_2O was added and placed on

stirrer for 30 min. The mixture was filtered with metal filter and pour filtrate into a large test tube. Analysis was conducted using ICP mass spectrometry.

Data Analysis

Data were analyzed by ANOVA with JMP 9.0 (SAS Institute Inc. Cary, NC). Mean separations were performed using a Fisher's protected LSD test at 5% probability level.

Results

Ni^{2+} Toxicity Symptoms

As Ni^{2+} concentration in leaf tissue increased the TQ decreased. Nickel toxicity symptoms including black lesions and overall chlorosis were exhibited in the 400, 800 and 1600 µM Ni^{2+} treatments (Figures 5.1-5.7).

Clipping Yield

Clipping yield was significantly influenced by the main effect of species and Ni^{2+} level. Diamond exhibited significantly greater clipping yield at 927.40 g m^{-2} compared to TifEagle at 629.40 g m^{-2} at the conclusion of the study. As Ni^{2+} level increased, clipping yield decreased (Figures 5.5 & 5.6). Under control treatments receiving no additional Ni^{2+}, overall clipping yield was 964.49 g m^{-2}. Clipping yields of 858.96, 704.20, and 585.95 g m^{-2} were exhibited for 400, 800 and 1600 µM Ni^{2+} respectively. Overall, 1600 µM Ni^{2+} treatments reduced clipping yield by 39% compared to the control. Under 1600 µM Ni^{2+} treatments Diamond exhibited a 34.1% reduction in clipping yield whereas TifEagle exhibited a 46.4% overall reduction.

N Concentration

N concentration in leaf tissue was significantly influenced by species (Table 5.3). TifEagle exhibited significantly higher N concentration than Diamond at 2.17 and 1.78 %DW respectively. Overall N concentration in leaf tissue was not affected by Ni^{2+} level.

Phosphorus Concentration

Species significantly affected P concentration in leaf tissue (Table 5.3). TifEagle exhibited significantly higher P contents than Diamond at 0.26 and 0.19 % DW respectively. All supplemental Ni^{2+} levels resulted in significantly lower P concentration in leaf tissue. Control plants exhibited 0.25, followed by 0.23, 0.21, and 0.21 for 800, 400 and 1600 µM Ni^{2+} levels respectively. All Ni^{2+} levels resulted in similar P concentrations and were not significantly different from each other.

Potassium Concentration

Diamond exhibited significantly higher K^{+} concentration in leaf tissue than TifEagle at 1.12 and 1.07 % DW respectively (Table 5.3).

Ni^{2+} Concentration

Nickel concentration in leaf tissue was significantly influenced by the main effects of species and Ni^{2+} level (Table 5.4). TifEagle exhibited significantly more Ni^{2+} in leaf tissue than Diamond at 257.56 and 130.09 mg kg^{-1} respectively. As Ni^{2+} level increased, the Ni^{2+} concentration in leaf tissue increased correspondingly. Plants not receiving supplemental Ni^{2+} exhibited overall Ni^{2+} concentration of 27.91 mg kg^{-1}, whereas Ni^{2+} concentrations of 101.28, 226.54, and 419.57 mg kg^{-1} were exhibited for 400, 800 and 1600 µM Ni^{2+} respectively.

Fe

The main effect of Ni^{2+} level significantly influenced Fe concentration in turfgrass leaf tissue at the conclusion of the study. Nickel concentrations ranged from 74.72 mg kg^{-1} in 800 µM Ni^{2+} treated plants; to 53.95 mg kg^{-1} in 1600 µM Ni^{2+} treated plants, a significant decrease (Table 5.4). However, control plants, not receiving any additional Ni^{2+} exhibited similar Fe concentrations as 800 and 1600 µM Ni^{2+} treated plants at 56.85, 63.27 and 53.95 mg kg^{-1} respectively. No clear reduction or trend could be observed.

Cu^{2+}

TifEagle exhibited significantly higher Cu^{2+} concentration in leaf tissue than Diamond at the conclusion of the study at 9.90 and 7.62 mg kg^{-1} respectively (Table 5.4). Nickel level significantly influenced Cu^{2+} concentration in leaf tissue also. Control plants receiving no additional Ni^{2+} exhibited the greatest Cu^{2+} concentration at 9.44 mg kg^{-1}, followed by 8.95 and 8.69 mg kg^{-1} for 800 and 400 µM Ni^{2+} treatments respectively. The lowest Cu^{2+} concentration was found in plants subjected to 1600 µM Ni^{2+} treatments at 7.96 mg kg^{-1}, significantly lower than all other treatments.

Zn^{2+}

TifEagle exhibited significantly higher Zn^{2+} concentration in leaf tissue than Diamond at the conclusion of the study at 24.87 and 23.47 mg kg^{-1} respectively. Nickel level influenced Zn^{2+} concentration in leaf tissue (Table 5.4). Zn^{2+} concentrations of 25.84, 24.71, 23.74 and 22.40 were exhibited for 1600, 800, 0, and 400 µM Ni^{2+} levels respectively. There was no clear trend between Ni^{2+} and Zn^{2+} concentration observed. It

might be possible that increasing Ni^{2+} level resulted in higher concentrations of Ni^{2+} in leaf tissue. Treatments of 1600 µM Ni^{2+} resulted in the greatest Zn^{2+} concentration.

Mn^{2+}

TifEagle exhibited significantly higher Mn^{2+}concentration in leaf tissue than Diamond at the conclusion of the study at 181.06 and 101.48 mg kg^{-1} respectively (Table 5.4). Nickel level affected Mn^{2+}concentration in leaf tissue. Concentrations of Mn^{2+}were reduced by the 400 µM Ni^{2+} level which exhibited significantly lower than all other Ni^{2+} levels at 126.04 mg kg^{-1}. Mn^{2+} concentrations of 152.63, 144.14, and 142.27 were displayed for 1600, 0, and 800 µM Ni^{2+} treatments respectively. No clear trend was observed for Ni^{2+}level and Mn^{2+} concentration in leaf tissue.

Mg^{2+}

TifEagle exhibited significantly higher Mg^{2+} concentration in leaf tissue than Diamond at the conclusion of the study at 2106.97 and 1313.44 mg kg^{-1} respectively. Nickel level significantly affected Mg^{2+} concentration in leaf tissue at the conclusion of the study (Table 5.4). All supplemental Ni^{2+} levels resulted in significantly lower Mg^{2+} concentration than control treatments. Plants not receiving additional Ni^{2+} exhibited Mg^{2+} concentrations of 1828.71 mg kg^{-1}, whereas all other treatments resulted in concentrations of approximately 1600 mg kg^{-1}, a significant reduction. Increasing Ni^{2+} caused reductions in Mg^{2+} concentration in leaf tissue, suggesting a similar uptake strategy.

Urease Activity

Urease activity was determined at the conclusion of the study. Both main effects (species and Ni^{2+} level) significantly influenced urease activity in leaf tissue (Table 5.2). TifEagle exhibited significantly higher urease activity than Diamond at the conclusion of the study at 106.49 and 81.30 μmol NH_4^+ min^{-1} g^{-1} respectively. Nickel level significantly influenced the urease activity in leaf tissue. As Ni^{2+} level increased so did the urease activity in leaf tissue. At 1600 μM Ni^{2+}, the average urease activity was 113.49 μmol NH_4^+ min^{-1} g^{-1}, followed by 102.0 and 93.74 μmol NH_4^+ min^{-1} g^{-1} for 800 and 400 μM Ni^{2+} levels respectively, which were not significantly different from each other. Control plants not receiving supplemental Ni^{2+} exhibited the lowest urease activity at 66.36 μmol NH_4^+ min^{-1} g^{-1}.

Amino Acid Content

Amino acid content was determined at the conclusion of the study. Diamond and TifEagle exhibited similar amino acid contents at 29.78 and 31 mg g^{-1} FW respectively. Nickel level was the only main effect that significantly influenced amino acid content in the leaf tissue (Table 5.2). 1600 μM Ni^{2+} treatments exhibited the greatest amino acid content in leaf tissue at 41.14 mg g^{-1} FW. Amino acid contents in leaf tissue began to decrease with 800 μM Ni^{2+} and control treatments resulting in similar contents at 30.60 and 25.93 mg g^{-1} FW respectively. The lowest amino acid contents in leaf tissue were found in 400 μM Ni^{2+} treated plants with an average content of 23.90 mg g^{-1} FW which wasn't statistically different from control treatments.

Discussion

Nickel toxicity decreased TQ, clipping yield, and influenced nutrient concentration in leaf tissue. Although both species experienced reductions in growth, TifEagle displayed greater reductions in clipping yield than Diamond when treated with 1600 µM Ni^{2+}. Under 1600 µM Ni^{2+} treatments Diamond exhibited a 34.1% reduction in clipping yield whereas TifEagle exhibited a 46.4% overall reduction compared to control treatments. However, TifEagle exhibited greater urease activity, N, P, and micronutrient concentration than Diamond. This result could be due to a concentration of nutrients in the reduced growth of the TifEagle leaf tissue.

Increasing Ni^{2+} level didn't correspond to a clear decrease in other micronutrient concentrations. Zinc concentrations in the leaf tissue actually increased with Ni^{2+} supplementation while other micronutrients didn't exhibit any trend (Table 5.4). This result further supports that theory of Ni^{2+} sharing the same uptake and transport mechanism of micronutrients including Cu^{2+} and Mg^{2+}.

Supplemental applications of Ni^{2+} stimulated urease activity in the leaf tissue over the course of the study which agrees with the findings of Krogmeier et al. (1991), Gerendas et al. (1998), and Gerendas and Sattelmacher (1999) with soybean, rice and spring rape respectively. Supplemental Ni^{2+} in the nutrient solution also increased the total amino acid pool in leaf tissue, presumably due to enhanced urease activity, which agrees with Gerendas and Sattelmacher (1999). Gerendas and Sattelmacher (1999) contributed the increase in amino acid content to storage and transport forms (Gln, Asn, Glu, and Asp). To further investigate this occurrence examination in specific amino acid

pools and N metabolites needs to be conducted. Witte (2011) suggested that N starvation and decreases in growth could be the result of elevated amino acid contents and urea as a sole N source, which is a possibility in this study. Elevated levels of total amino acids in leaf tissue might decrease the plant's need to absorb and assimilate foliar applied urea N.

Visual signs of Ni^{2+} toxicity were recorded throughout the study. Critical toxicity levels in plants are in the range of > 10 mg kg^{-1} dry weight for sensitive, and > 50 mg kg^{-1} dry weight in moderately tolerant species (Marschner, 1995). Under 1600 µM Ni^{2+} treatments, overall concentrations of Ni^{2+} in leaf tissue were 419.57 mg kg^{-1}. At this concentration a reduction in growth and TQ was displayed and Ni^{2+} toxicity was likely the cause. Plants receiving 400 and 800 µM Ni^{2+} also displayed reductions in growth and overall TQ while control plants exhibited no toxicity symptoms and overall Ni^{2+} concentrations of 27.91 mg kg^{-1}. Nickel concentrations for 400 and 800 µM treatments were 101.28 and 226.54 respectively, suggesting that the critical Ni^{2+} toxicity level in warm season turfgrass begins at a range >25 mg kg^{-1}.

Conclusions

This is the first study examining Ni^{2+} toxicity and urea N metabolism of warm-season turfgrasses. Stimulation of N metabolism was displayed through increases in urease activity and amino acid content, however, increased Ni^{2+} concentration in leaf tissue led to symptoms of toxicity, changes in nutrient status, reduction in growth, decreases in TQ, and leaf necrosis. Due to the responses of Diamond and TifEagle under Ni^{2+} stress the critical Ni^{2+} toxicity level in begins at a range >25 mg kg^{-1}. Nickel

concentrations in leaf tissue greater than 25 mg kg^{-1} caused reductions in growth and symptoms of toxicity.

This study examined Ni^{2+} toxicity using an 85:15 (sand: peat) root-zone which is commonly used on putting surfaces throughout the world. The previous chapter examined Ni^{2+} supplementation under solution culture and moderate salinity stress. Different responses in nutrient status and urea N metabolism within plants were displayed between these two studies which is common when comparing between soil and solution culture. Under solution culture, amino acid levels were greater in Diamond than TifEagle under moderate Ni^{2+} supplementation. However, TifEagle displayed greater overall N concentration in leaf tissue than Diamond. In this experiment, a more direct examination of Ni^{2+} supplementation and toxicity was displayed without the additional influence of moderate salinity stress. TifEagle exhibited greater N, P, and micronutrient concentrations in leaf tissue than Diamond under Ni^{2+} stress but displayed a greater reduction in growth. This finding could be a result of a concentration of the mineral nutrients in the reduced shoot growth of TifEagle.

Future research examining Ni^{2+} nutrition and toxicity needs to be conducted to determine Ni^{2+} requirement under several N sources and multiple turfgrasses. Additionally, research needs to be conducted to examine if Ni^{2+} supplementation aids in foliar recovery of applied urea N and if foliar applications of Ni^{2+} can be made. Lastly, research should focus on Ni^{2+} requirement of turfgrasses along with long term ecological, physiological, and environmental implications of Ni^{2+} supplementation in turfgrass environments.

Table 5.1. Stock solutions and concentrations for nutrient solutions based on Hoagland and Arnon (1950).

Nutrient	Stock Solution Concentration
G. Magnesium sulfate, $MgSO_4$ *7H_2O	**1M**
H. Potassium sulfate, K_2SO_4	**0.6 M**
I. Magnesium sulfate, $MgSO_4$	**1M**
D. Calcium Phosphate Monobasic, CaH_2PO_4	**0.05M**
E. Micronutrient Stock	**(g/L)**
Boric Acid, H_3BO_3	**2.86**
Manganese chloride, $MnCl_2$*4H_20	**1.81**
Zinc sulfate, $ZnSO_4$ * 7H_2O	**0.22**
Copper sulfate, $CuSO_4$ * 5 H_2O	**0.08**
Molybdic acid, MoO_3*H_2O	**0.02**
F. Fe (Sequestrene)	**21.0**
G. Nickel chloride, $NiCl_2$*6$H2_O$	**0.81**

Minus N Solution: The nutrient solution was prepared using the following mL of stock solution per liter of final solution: 5ml of stock B; 2 ml of stock A; 20 ml of stock D; 1 ml of stock E; 1 ml of stock F.

Ni Treatments: The nutrient solution was prepared using the following mL of stock solution per liter of final solution: 0 µM : 0 ml/L;400 µM : 25ml/L;800 µM : 50ml/L;1600 µM : 100ml/L

Table 5.2. Urease activity and amino acid content in leaf tissue of 'Diamond' zoysiagrass and 'TifEagle' bermudagrass as influenced by Ni level, and species in Clemson University Greenhouse Research Complex during 2012.

Main effects	Urease	Amino Acid
	-µmol NH_4^+ min^{-1} g^{-1}-	------mg g^{-1}-------
Ni Level µM (Ni)		
Control	66.3	25.9
400	93.7	23.9
800	102.0	30.6
1600	113.49	41.1
$LSD_{0.05}$	12.60	5.6
Species (SP)		
Diamond	81.30	29.7
TifEagle	106.49	31.0
	ANOVA	
Source of variation		
Ni	***	***
SP	***	NS
Ni*SP	**	NS

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

Table 5.3. Macronutrient concentration (%DW) in leaf tissue of 'Diamond' zoysiagrass and 'TifEagle' bermudagrass as influenced by Ni level, and species in Clemson University Greenhouse Research Complex during 2012.

Main effects	Nitrogen	Phosphorus	Potassium
	----------% DW----------		
Ni Level µM (Ni)			
Control	2.05	0.25	1.13
400	1.96	0.21	1.09
800	1.91	0.23	1.07
1600	2.00	0.21	1.11
$LSD_{0.05}$	NS	0.01	NS
Species (SP)			
Diamond	1.78	0.19	1.07
TifEagle	2.17	0.26	1.12
	ANOVA		
Source of variation			
Ni	NS	***	NS
SP	***	***	**
Ni*SP	NS	NS	*

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

Table 5.4. Micronutrient concentration (mg kg^{-1}) in leaf tissue of 'Diamond' zoysiagrass and 'TifEagle' bermudagrass as influenced by Ni level and species in Clemson University Greenhouse Research Complex during 2012.

Main effects	Ni	Fe	Cu	Zn	Mn	Mg
	---mg kg^{-1}---					
Ni Level µM (Ni)						
Control	27.91	56.85	9.44	23.74	144.14	1828.71
400	101.28	63.27	8.69	22.40	126.04	1683.30
800	226.54	74.72	8.95	24.71	142.27	1685.89
1600	419.57	53.95	7.96	25.84	152.63	1642.91
$LSD_{0.05}$	49.35	15.45	0.65	1.65	14.50	105.82
Species (SP)						
Diamond	130.09	57.52	7.62	23.47	101.48	1313.44
TifEagle	257.56	66.52	9.90	24.87	181.06	2106.97
			ANOVA			
Source of variation						
Ni	***	*	**	**	*	*
SP	***	NS	**	*	***	***
Ni*SP	***	**	NS	***	***	*

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

Figure 5.1. Nickel toxicity symptoms on leaf tissue of Diamond (L) and TifEagle (R) under1600 µM Ni treatments.

Figure 5.2. Nickel toxicity symptoms on leaf tissue of Diamond (L) and TifEagle (R) under 800 µM Ni treatments.

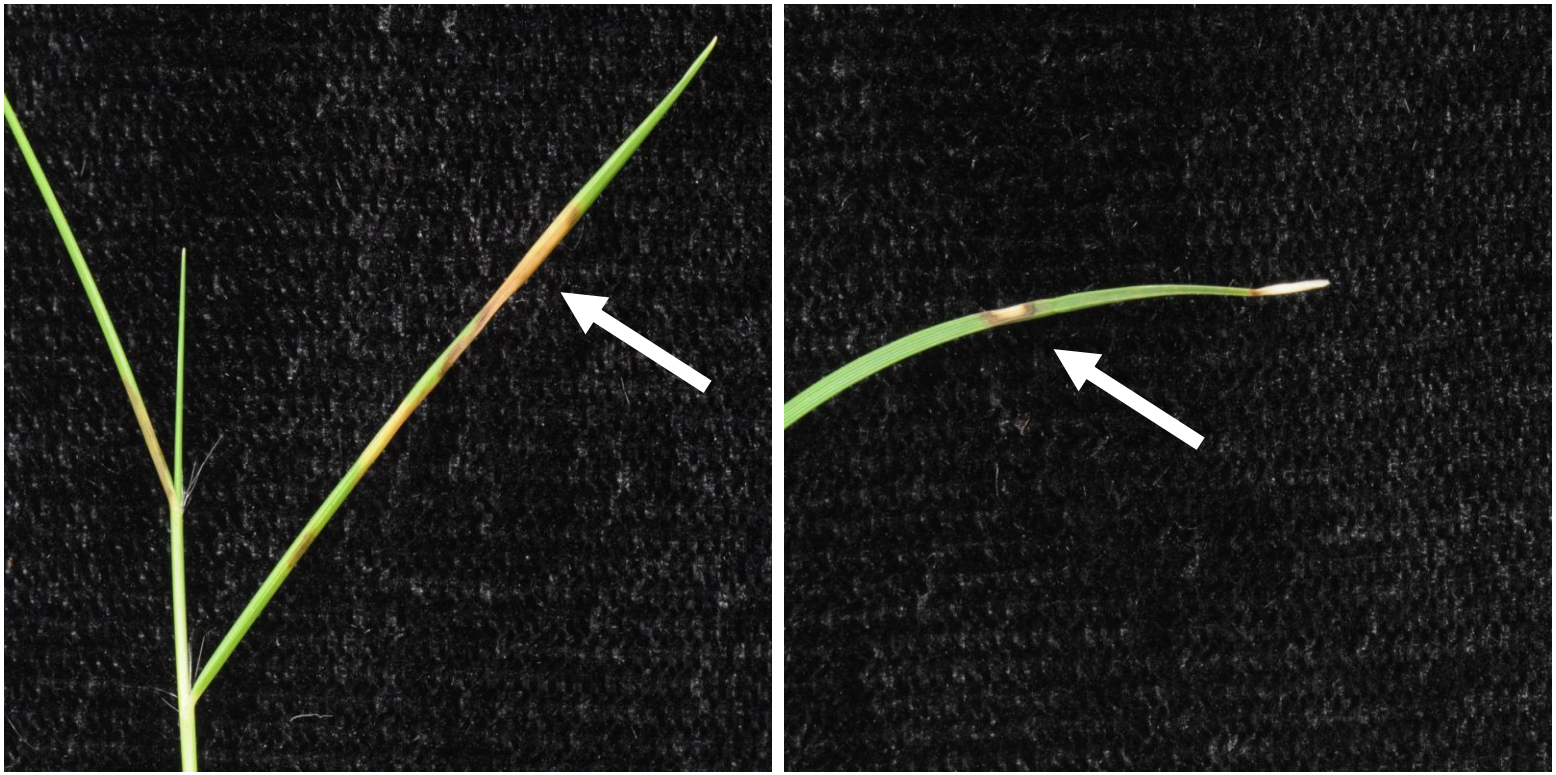


Figure 5.3. Nickel toxicity symptoms on leaf tissue of Diamond (L) and TifEagle (R) under 400 µM Ni treatments.

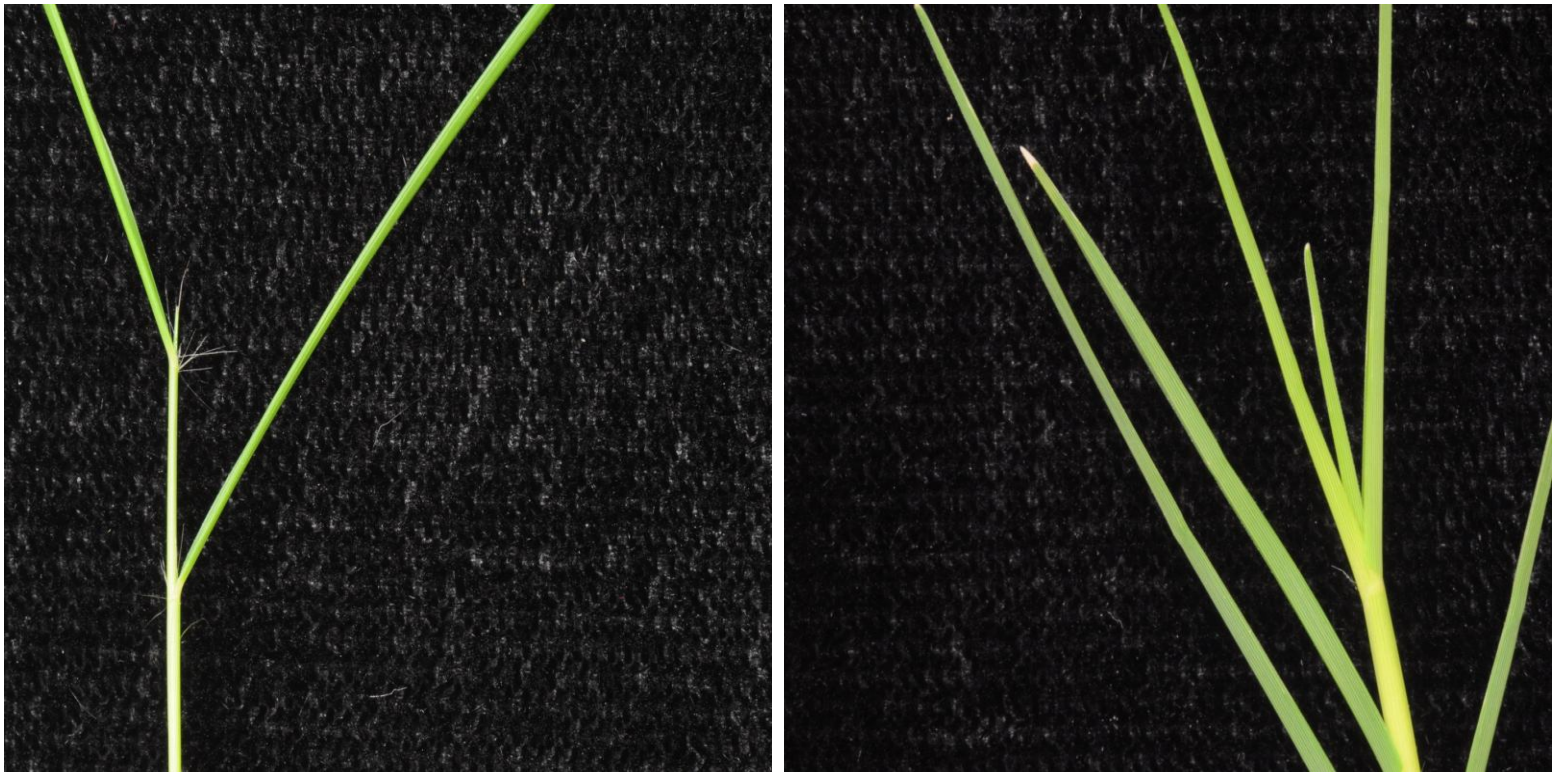


Figure 5.4. Nickel toxicity symptoms on leaf tissue of Diamond (L) and TifEagle (R) under control treatments.

Figure 5.5. TifEagle bermudagrass growth reduction under five Ni^{2+} levels (Control, 400, 800, 1600 µM Ni^{2+})

Figure 5.6. Diamond zoysiagrass growth reduction under five Ni^{2+} levels (Control, 400, 800, 1600 µM Ni^{2+})

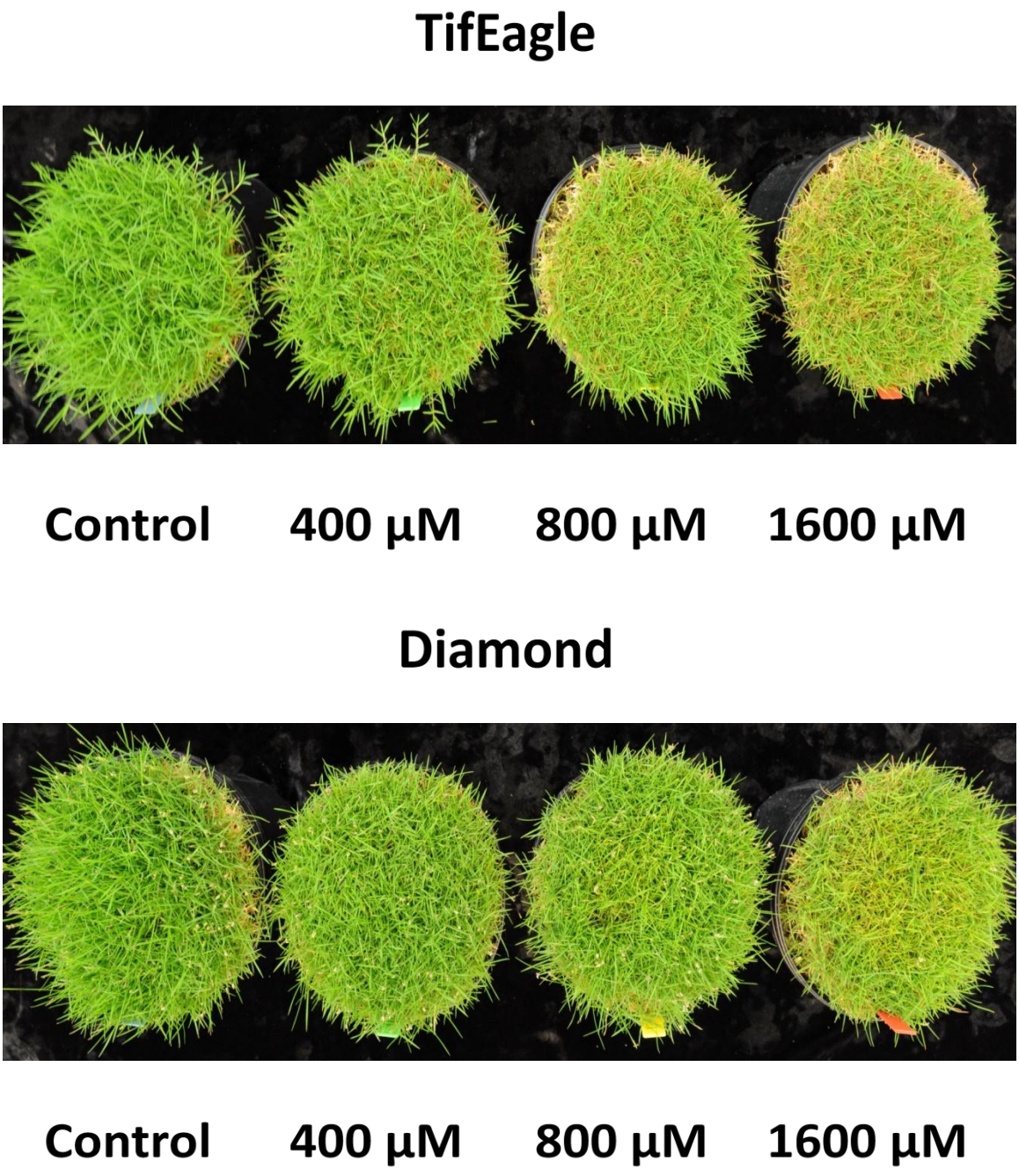


Figure 5.7. Diamond zoysiagrass and TifEagle TQ under five Ni^{2+} levels (Control, 400, 800, 1600 µM Ni^{2+}) at the conclusion of the study in the Clemson University greenhouse research complex during 2012.

CHAPTER VI

ROOT AND FOLIAR ^{15}N UREA RECOVERY OF THREE WARM-SEASON TURFGRASS SPECIES

Introduction

Granular and foliar fertilization are the two most common ways to apply mineral nutrients to turfgrasses. Granular fertilization targets root uptake while foliar fertilization targets nutrient uptake through leaf tissue. Greater amounts of nutrients can be supplied through granular fertility programs, however many benefits have been found with foliar programs (Liu et al., 2008). Although commonly practiced in turfgrass management, little is known about the uptake and assimilation of foliar applied fertilizers.

Labeled ^{15}N urea was utilized to examine the uptake, recovery, and distribution of foliar and root applied fertilizers. Typically, research projects examining the differences between root and shoot uptake of various N sources commonly employ applications of granular fertilizers. Picchioni and Quiroga-Garza (1999) found that 'TifGreen' bermudagrass exhibited the greatest loss of applied ^{15}N under soluble urea fertilization, reporting that plants under urea fertility regimes exhibited the smallest N yield in leaf tissue compared to ammonium sulfate and ammonium nitrate fertility programs. To more accurately examine the differences between root and leaf uptake, soluble urea N was delivered directly to the root zone with a large syringe or by a CO_2 pressurized sprayer. By utilizing this methodology, actual differences between uptake and movement through the plant could be more thoroughly assessed.

To further compare the two common delivery methods of urea N this study was conducted to 1) assess ^{15}N recovery of three popular warm season turfgrass species following foliar and root applications of urea nitrogen, 2) determine the effect of fertility regime on ^{15}N recovery, and 3) establish fertilization recommendations for optimal fertilizer recovery/efficiency.

Materials and Methods

Six 20.32 cm plugs of 'Mini-Verde' bermudagrass, and Diamond zoysiagrass, and Seadwarf seashore paspalum were harvested from research plots at the Cliffs Research Facility (Marietta, SC) in December 2009. The plugs were thoroughly washed of excess root zone mix and transferred into 1 gallon plastic pots filled with 85%:15% sand: peat v:v (USGA Green Section Staff, 1993) and irrigated until free drainage occurred. The pots were then transferred into the Greenhouse Research Complex at Clemson University. The turfgrasses were allowed to acclimatize establish before further management was conducted. After a week in the greenhouse, fertilization took place using Progressive Turf Products (Ball Ground, GA) 10N-1.3P-4.2K foliar fertilizer. Fertilization took place on December 18th 2009 and January 29th 2010 delivering 9.77 kg^{-1}ha^{-1} at 560 L^{-1}ha^{-1}. Two preventative applications of Daconil Ultrex (chlorothalinil) were done. Pots were mown to a uniform height (1.27 cm) on February 25th 2010.

Treatment Procedure

Foliar applications of labeled urea @ 2% enrichment were applied at a rate of 9.77 kg ha^{-1} with a pressurized Co_2 backpack sprayer calibrated to deliver 560 L ha^{-1}. To prepare the ^{15}N solution, 3.93 g of 2% labeled urea-N (Icon Isotopes, Summit, NJ) was

mixed in 114.1 ml ddi water and thoroughly mixed in a 1L volumetric flask. Root applications of urea N were prepared by diluting 1.94 g labeled urea @ 2% enrichment into 1,000 ml H_2O. Twenty ml of this solution was applied to five different areas in the root zone with a 60 ml carpet syringe (Ideal Instruments HD AC14) to ensure uniform application across each experimental unit. Total N delivered was determined in both nutrient solutions (root and foliar). Samples were collected in Petri dishes and thoroughly washed with 100 ml de-ionized distilled H_2O before being tested for total N by Kjeldahl digestion.

Sample Harvest

Turfgrass clippings, thatch and roots were harvested 8 hours after treatments were applied from three replicate pots of each cultivar using a 10.8 cm soil probe. Leaf tissue was sampled using electric clippers (Wahl) and placed into coin envelopes and dried at 80°C. The remaining sample including root material and thatch were divided, separated, and placed into labeled paper bags and dried at 80° C until further analysis. Soil samples were taken from each pot, placed in paper bags and dried at 80° C until further analysis.

^{15}N Analysis

Analysis of isotopic ^{15}N in tissue samples and fertilizer applied was determined at the University of Illinois at Urbana-Champaign using the automated Rittenburg technique (Mulvaney et al., 1990), on a Nuclide/MAAS 3-60-RMS double mass spectrometer (Nuclide Corporation, Bellefonte, PA). Recovery of 15N was determined by the following: Recovery of fertilizer N in any fraction is calculated as:

$$100 \times (S - B)/ (F - B)$$

Where S is the atom % ^{15}N for the sample under analysis, F is the atom % ^{15}N for the fertilizer used, and B is the atom % ^{15}N with no addition of labeled N (sometimes assumed to be 0.3663 at. % ^{15}N, but likely closer to 0.370 at. % ^{15}N and preferably determined for a control soil with no addition of labeled fertilizer). Further calculations were made to determine total ^{15}N recovery that was derived from the labeled source.

Data Analysis and Experimental Design

The study was completely randomized design with three replications and a factorial treatment arrangement with three turfgrass species and two fertility regimes. Data was analyzed by ANOVA with JMP 9.0 (SAS Institute Inc. Cary, NC). Mean separations were performed using a Fisher's protected LSD test at 5% probability level.

Results

^{15}N Recovery

Recovery of ^{15}N labeled urea was measured 8 hours after application in clippings, thatch, roots and soil. Results of ^{15}N recovery will be determined by each respective plant part, total recovery, and recovery in soil

Leaf tissue

Recovery of ^{15}N labeled urea in leaf tissue was significantly influenced by fertility regime. When applied as a foliar application, recovery at 8 hours was 30.94% whereas root applied urea resulted in a recovery of only 9.17% (Figure 6.1).

Thatch

Recovery of ^{15}N labeled urea in thatch was significantly influenced by fertility regime. When applied as a root application, recovery at 8 hours was 18.73% whereas

foliar applied urea resulted in a recovery of only 2.85% (Figure 6.2). Recovery in thatch was also influenced by species, and an interaction between fertility regime*species. Diamond exhibited recoveries of 14.11% in thatch tissue, which was significantly higher than the recovery in Seadwarf at 7.03%. MiniVerde exhibited similar levels of labeled urea in thatch tissue as MiniVerde and Seadwarf at 11.25%, which was not statistically different from either species. The interaction of fertility regime*species further displays the influence of fertility regime on ^{15}N thatch recovery. All root applications of urea N resulted in significantly higher recoveries in thatch tissue than foliar treatments. Within root treatments, Diamond and MiniVerde exhibited similar 15N recoveries at 24.53 and 20.82%, which were both significantly increased over recovery in Seadwarf at 10.84%.

Root tissue

Recovery of ^{15}N labeled urea in root tissue was significantly influenced by fertility regime. When urea was applied as a root application, recovery at 8 hours was 19.48% whereas foliar applied urea resulted in a recovery of only 3.76% in root tissue (Figure 6.3).

Total ^{15}N recovery and soil ^{15}N retention

Overall ^{15}N recovery in plant tissue was not significantly influenced by fertility regime or species. Overall recovery for foliar fertilization was 37.56% compared to 47.39% for root applications of urea (Figure 6.4). Recoveries of 47.62%, 44.66%, and 35.14 % were exhibited for Seadwarf, Diamond, and MiniVerde respectively (Figure 6.5). At 8 hours after treatments were made, significantly more labeled urea was found in the soil of plants receiving root applications. ^{15}N recovery of 5.55% was exhibited for

root applications whereas foliar applications of urea resulted in only 0.83% recovery in the soil (Figure 6.6).

Discussion

Total plant recovery of ^{15}N labeled urea derived from fertilizer was not significantly different in either fertility regime or species tested. Although not statistically different, root applications of urea N resulted in 10% higher total ^{15}N recovery than foliar treatments at 8 hours after application. Stiegler et al. (2011) found overall foliar absorption ranging from 38-62% for TifEagle bermudagrass under field conditions, which is slightly greater than foliar recovery in the present study at 37.56%. In addition, foliar absorption values ranging from 30-60% were found by in many cool-season turfgrass species (Bowman and Paul, 1989; Bowman and Paul, 1990; Bowman and Paul, 1992). Henning et al. (2009) quantified foliar urea-N uptake of 25-30% on creeping bentgrass within 6 hours after application. There were also differences in total plant recovery across species although not statistically significant. MiniVerde displayed the lowest total ^{15}N recovery at 8 hours at 35.14%, which was much lower than Diamond and Seadwarf at 44.66% and 47.62% respectively. Differences in ^{15}N recovery displayed in these studies could be due to environmental conditions, species, methodology, and sampling techniques.

Stiegler et al. (2011) found that foliar applications of 1.25 g N m^{-2} were less efficient than 0.5 g N m^{-2} when expressed as percent of applied N in creeping bentgrass and bermudagrass putting surfaces. Foliar and root applications of 0.97 g N m^{-2} were used in this study. It is possible that reducing the N rate could increase total recovery in

the turfgrass. Due to the possibility that increased N rates could result in precipitation of the N out of the spray droplet on the leaf tissue and therefore reduce total absorption (Stiegler et al., 2011).

Recovery of labeled urea in each plant part was significantly influenced by fertility regime, and was anticipated. Foliar applications of urea resulted in higher recovery in leaf tissue while root applications resulted in elevated ^{15}N recoveries in root tissue. Total ^{15}N recovery was also significantly higher in root applications than foliar fertilization. In addition to recovery in specific plant tissue, root applications of urea N resulted in significantly higher ^{15}N retention in soil than foliar applications, however overall recovery of ^{15}N derived from fertilizer was higher in root treatments. The 10% overall reduction in ^{15}N recovery for foliar treatments could be due to a number of factors, including volatilization. However, foliar fertilization resulted in significantly less ^{15}N urea in the soil. This is beneficial due to the problems with fertilizer loss, leaching, ecological implications, etc.

The disparity, although not statistically different, in total ^{15}N recovery due to fertility regime is worth examining more closely. ^{15}N labeled urea retained in the soil (5.55%) 8 hrs after root applications has the ability to be taken up by the plant potentially increasing the overall recovery over time. Foliar treatments resulted in ^{15}N recovery in the soil of <1%. Leaching and volatilization losses were not quantified for this study, and account for the large portion of N lost when sampling took place.

Conclusions

Foliar fertilization has become commonplace in turfgrass management due to the ability of managers to tank mix fertility programs with pesticides to correct plant deficiencies while controlling turfgrass pests. Based on our results, foliar and root applications of urea N resulted in overall recovery in plant tissue of 37 and 47%. Significant fertilizer loss (up to 63% of applied N) was exhibited that could be due to a number of reasons. More research is necessary to increase the uptake of applied urea N in foliar and root delivery methods.

Many management strategies have been employed to improve fertility uptake and retention in turfgrass ecology. In addition to traditional management strategies, one approach might examine and potentially modify the turfgrass cuticle to improve foliar penetration of soluble N sources. In addition, Stiegler et al. (2011) suggests using low application rates (< 0.5 g N m^{-2}), waiting several hours to maximize foliar absorption, and watering in residual unabsorbed N off the canopy into the rootzone will maximize uptake efficiency. Further examination of N rates and carrier volume could prove beneficial in increasing foliar applied urea N recovery.

Secondly, urea must be hydrolyzed by the nickel dependent enzyme urease before it can be incorporated into organic N containing compounds. Through examination of urea N metabolism and hydrolysis, it might be possible to improve foliar and root recovery. Further research into N metabolism needs to be conducted to examine reduce fertility inputs while increasing plant uptake and assimilation. Long term (>8hours)

recovery, partitioning and uptake efficiency needs to be conducted to further examine the differences in delivery method of urea N in warm-season turfgrass management.

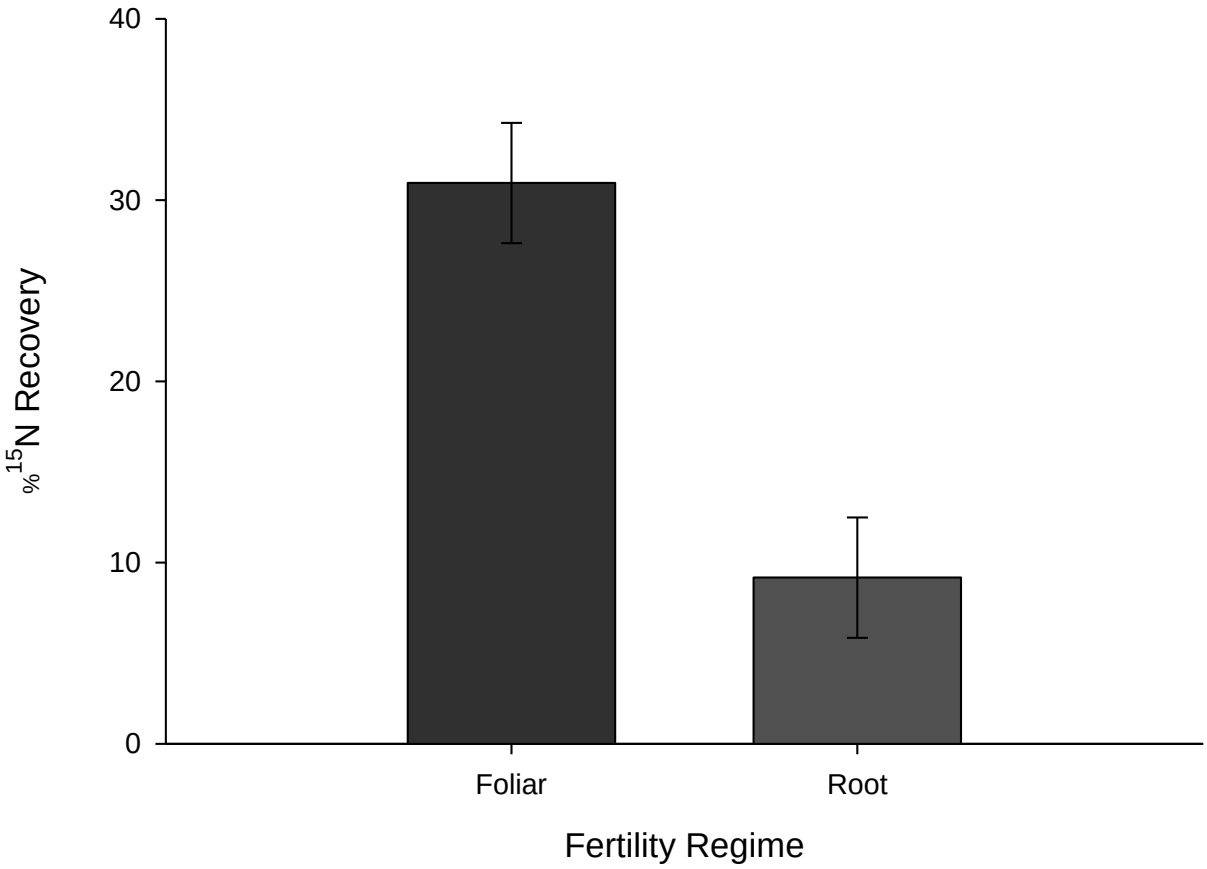


Figure 6.1. Overall % ^{15}N recovery in leaf tissue following foliar and root applications of labeled urea N at the Clemson University greenhouse research complex 8 hours after treatments were initiated. Means were separated at P≤0.05 by protected LSD.

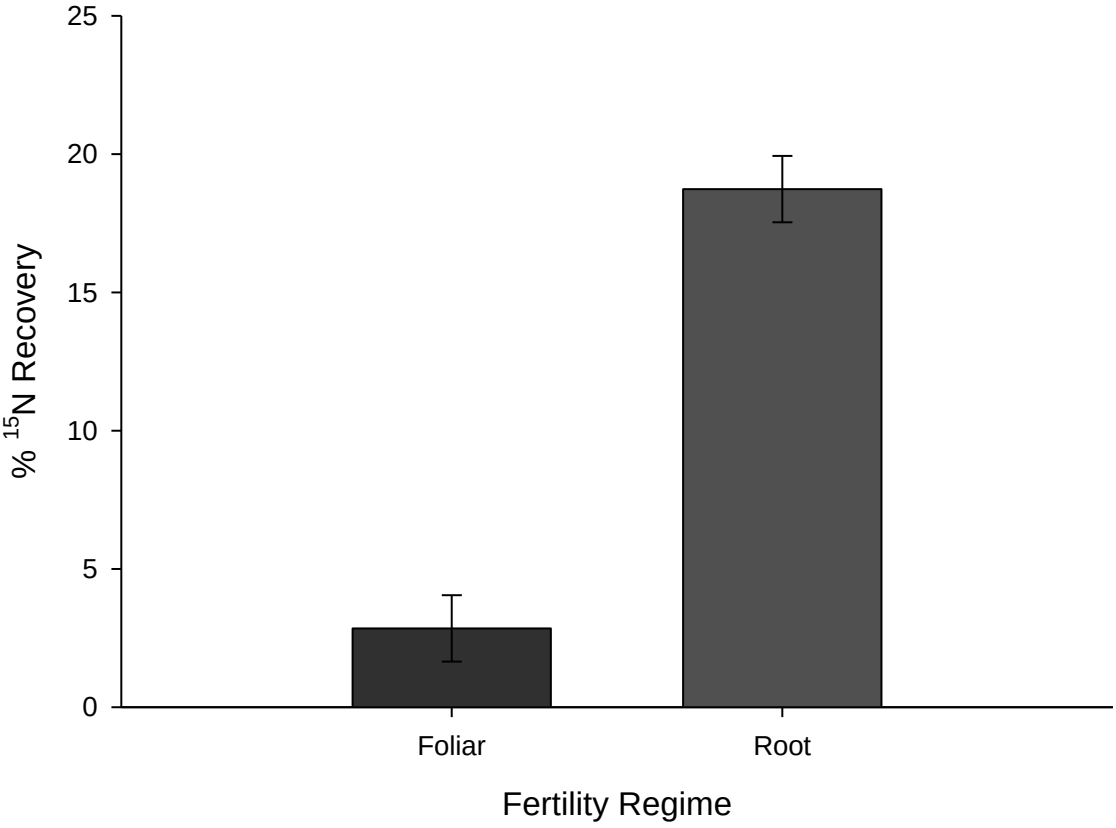


Figure 6.2. Overall % ^{15}N recovery in thatch tissue following foliar and root applications of labeled urea N at the Clemson University greenhouse research complex 8 hours after treatments were initiated. Means were separated at P≤0.05 by protected LSD.

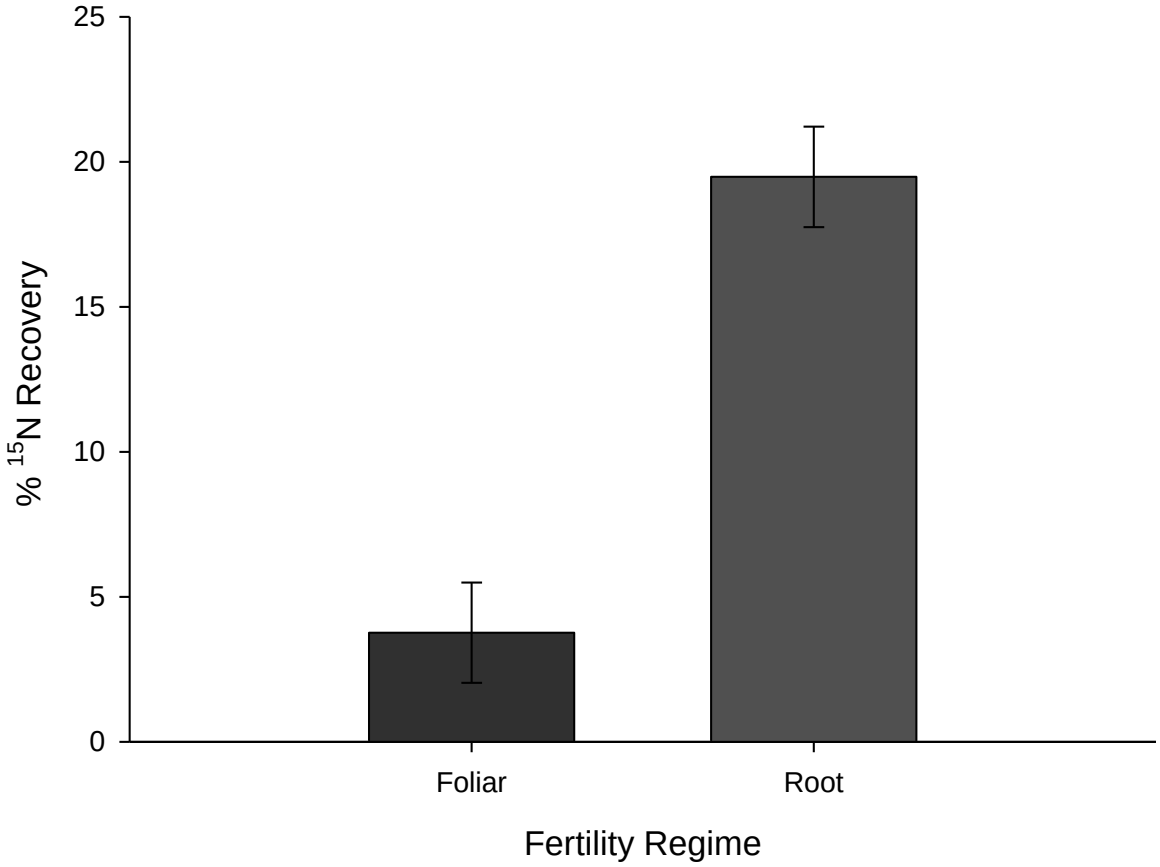


Figure 6.3. Overall % ^{15}N recovery in root tissue following foliar and root applications of labeled urea N at the Clemson University greenhouse research complex 8 hours after treatments were initiated. Means were separated at P≤0.05 by protected LSD.

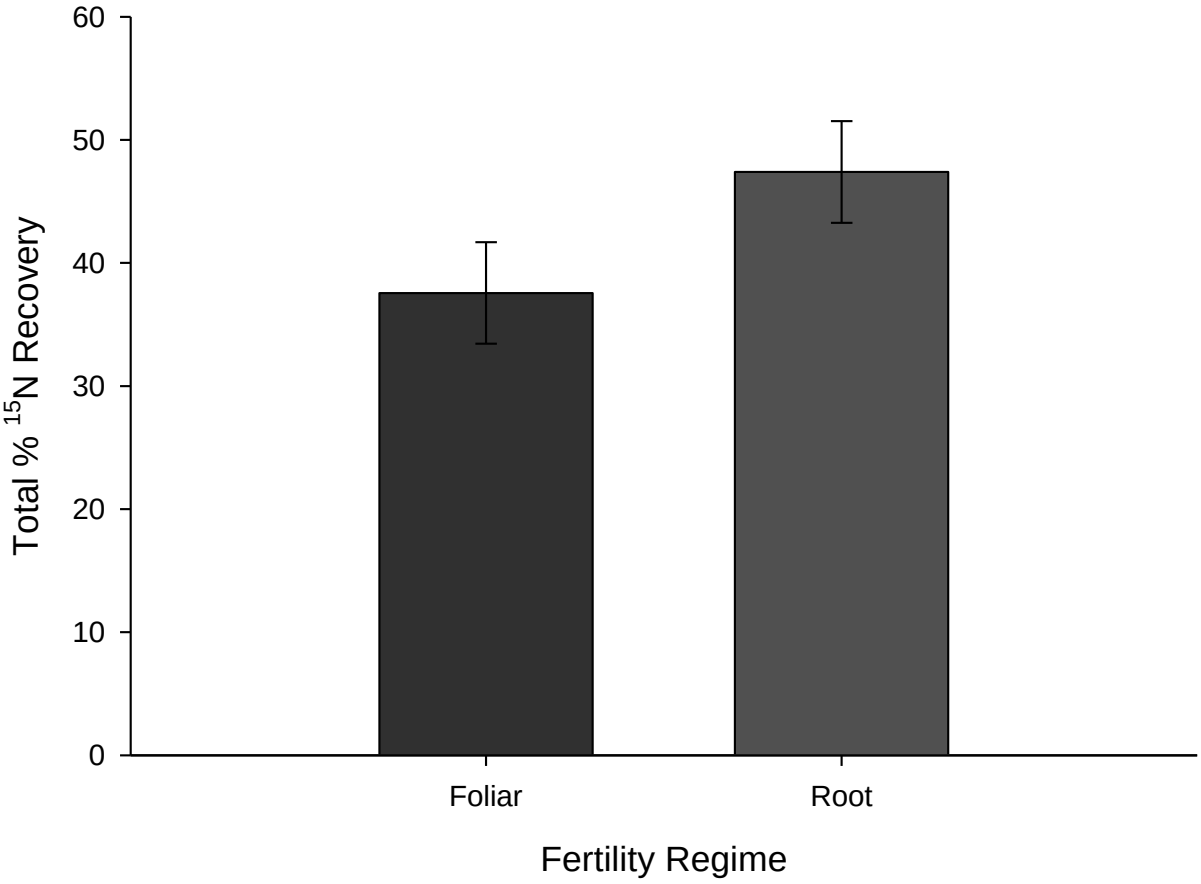


Figure 6.4. Total % ^{15}N recovery following foliar and root applications of labeled urea N at the Clemson University greenhouse research complex 8 hours after treatments were initiated. Means were separated at P≤0.05 by protected LSD.

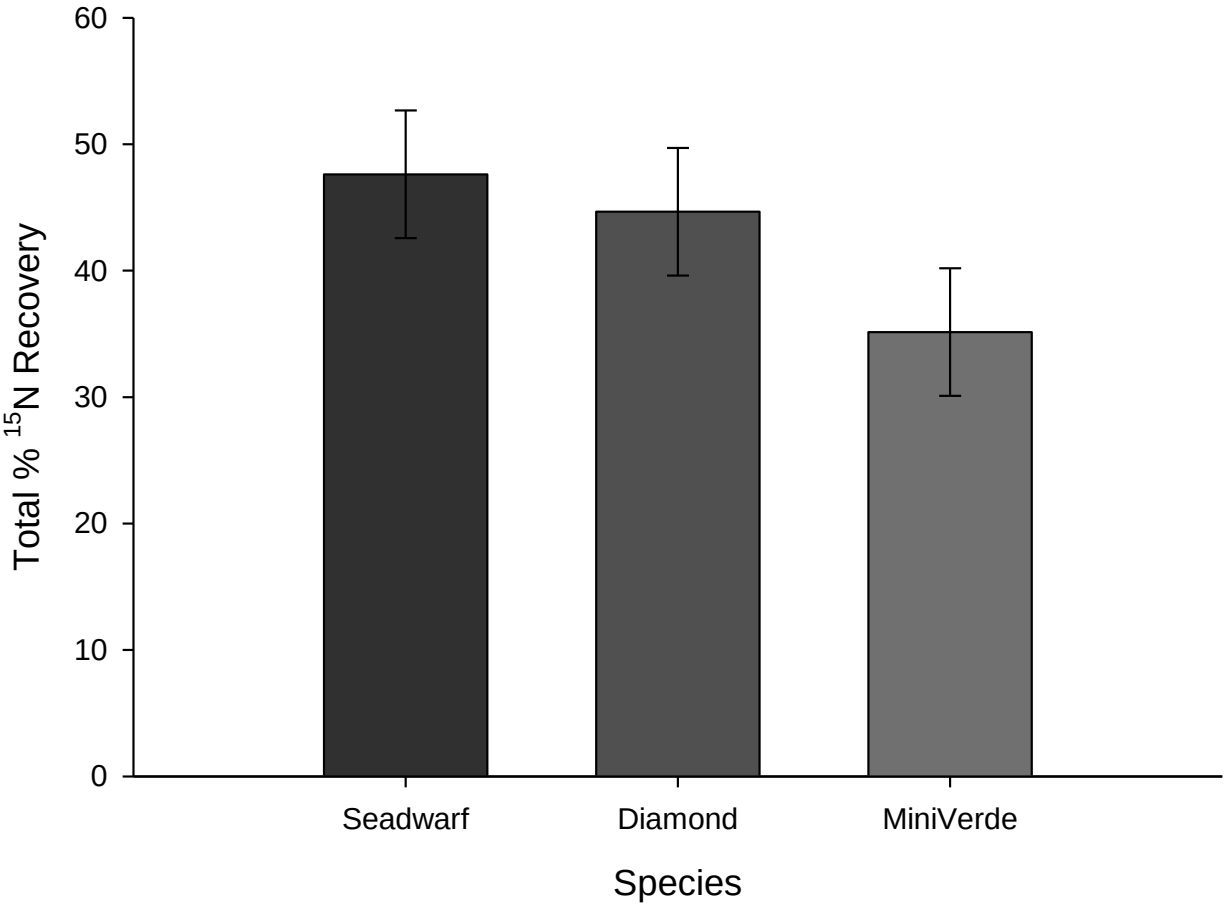


Figure 6.5. Total % ^{15}N recovery of Diamond, MiniVerde and Seadwarf at the Clemson University greenhouse research complex 8 hours after treatments were initiated. Means were separated at P≤0.05 by protected LSD.

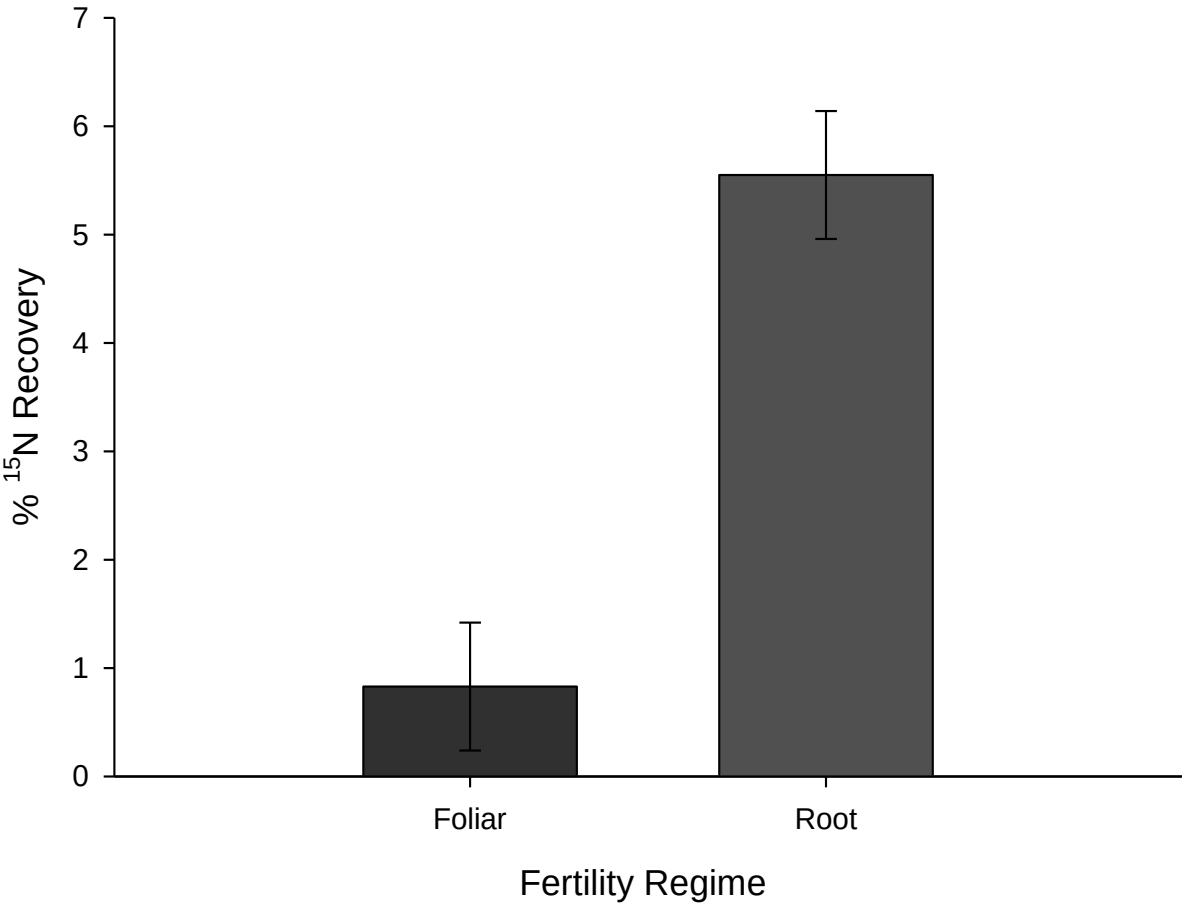


Figure 6.6. Total % ^{15}N recovery in soil following foliar and root applications of labeled urea N at the Clemson University greenhouse research complex 8 hours after treatments were initiated. Means were separated at P≤0.05 by protected LSD.

CHAPTER VII

EFFECTS OF VARYING RATES OF NITROGEN AND TRINEXAPAC-ETHYL ON PUTTING GREEN PERFORMANCE OF 'DIAMOND' ZOYSIAGRASS IN THE TRANSITION ZONE

Introduction

Turfgrasses managers in the Southern transition zone and further south have had a difficult choice when deciding what turfgrass species to establish on putting greens. The choices have commonly been between creeping bentgrass and dwarf bermudagrass varieties. Developments in turfgrass genetics and breeding has led to ultradwarf bermudagrass varieties that are more heat and drought tolerant than their bentgrass counterparts (McCarty, 2011). However, these relatively new ultradwarf varieties have their limitations too, including shade and cold tolerance. To further complicate the choice, a new turfgrass option for putting greens are fine leafed zoysiagrass cultivars including Diamond (Menchyk et al., 2012).

Diamond is a fine textured warm-season turfgrass with excellent shade, salt, and cold tolerance. Atkinson et al. (2011) found that Diamond zoysiagrass could be grown successfully at putting green mowing heights with the application of trinexapac-ethyl (TE) under 60% shade. The popularity of Diamond zoysiagrass has grown in the past few years with many fairway, tee, and collar installations on golf courses. Recently, turfgrass managers in the Southeast USA have begun to utilize Diamond on putting greens. However, little is known about the management, health, and performance of fine

leafed zoysiagrass cultivars in such a scenario. This study was conducted to examine the management of Diamond zoysiagrass in a putting green scenario.

The objective of this study was to evaluate the influence of foliar applied ammonium nitrate, and trinexapac-ethyl on Diamond zoysiagrass putting green performance by (1) measuring ball roll distance (speed), (2) determining surface firmness, (3) measuring thatch accumulation (4) examining tissue nutrient concentrations, and (5) measuring turf quality.

Materials and Methods

Plant Material

Diamond zoysiagrass was established by sprigs (New Life Turf) on July 8, 2008 at the Cliffs Research Facility in Marietta, SC. The plots were constructed as USGA specification greens with an 85:15 root-zone and subsurface drainage (USGA green section, 1993). For the duration of the growing season the plots were mowed daily at 3.2mm. During winter dormancy, the plots were not mowed, covered, over-seeded, or painted.

Treatment

Treatments of 4.9, 9.8, and 14.7 kg N ha^{-1} ammonium nitrate were applied foliarly. Trinexapac- ethyl (TE; Primo MAXX (EC) 11.3% ai. Syngenta Chem. CO., Greensboro, NC.) was applied at 0 L a.i ha^{-1} or 0.017 L a.i ha^{-1}. Treatments in 2009 were initiated on July 28th and finished on September 10th. In 2010, treatments began on May 20th and terminated on August 25th. For the duration of the study in 2009 total N treatments lasted 7 weeks totaling – 34.3, 68.6, and 102.9 kg N ha^{-1}, respectively, for the

three treatments. For the duration of the study in 2010 total N treatments lasted 15 weeks resulting in 73.5,147, and 220.5 kg N ha^{-1}, respectively, for the three treatments. TE applications for 2009 and 2010 were the same and totaled 0.120 L ai/ha starting in July and ending in September at a biweekly rate of 0.017 L a.i ha^{-1}. Treatments were applied using a CO_2 pressurized backpack sprayer delivering 561 L ha^{-1}. Plots measuring 0.91 x 3.66 M were arranged in a completely randomized design with three replications.

Parameters Measured

Weekly readings of turf quality were performed. Color, texture, density, and playability were factored into the ratings (1-9 >6 acceptable, where 1=dead turf and 9 = perfect turf). Thatch was measured monthly, coinciding with Trufirm™ readings. Four subsamples were taken in each plot corresponding to the four firmness readings with a soil sampler. Each thatch sample was oven-dried at 72 C^o for 48 hours. Loss on ignition (thatch ash-free) data was determined as follows. After drying the samples, shoot and excess root materials were excised and a dry weight of the remaining thatch was recorded. Samples were then ashed at 525 C^o for 2 hours using a muffle furnace (Omegalux LMF-A550). The ashed weight was recorded and the total organic material was determined. Surface firmness was measured using Trufirm™ (USGA) monthly. Trufirm measures the penetration in fractions of an inch. Four subsamples were taken in each plot and averaged. Ball rolling distance was measured using a Stimpmeter (USGA) weekly. Means were analyzed using JMP Version 9 (Cary, NC).

Results

Turf Quality

Turf quality was significantly influenced by the main effect of N fertility rate. 14.7 kg N ha^{-1} treatments exhibited significantly higher TQ than plots receiving 4.9 kg N ha^{-1} and 0 kg N ha^{-1} weekly. Treatments of 14.7 and 9.8 kg N ha^{-1} exhibited similar TQ during the study at 6.99 and 6.96 respectively. Only control treatments displayed TQ values below the acceptable level of 6.0 (Figure 7.1). TE had no main effect on TQ throughout the study. TQ was significantly influenced by the month in which it was recorded, due to fluctuations in seasonal turfgrass growth. Differences in overall TQ were exhibited across the 8 months, however only November 2010 exhibited overall TQ values below the minimally acceptable level. The main effect of year was significant in turf quality, with 2010 exhibiting significantly lower values. This result was possibly due to the duration of treatments during 2010 season, and can be more fully examined in the N level*year interaction which was highly significant. During 2009, all of the N levels resulted in very similar TQs ranging from 6.57 to 7.01. During 2010, the TQ values were more varied, ranging from 4.63 for the control treatments, to 7.40 for 14.7 kg N ha^{-1} treatments. A month*year interaction was also seen, where every month except September in 2009 resulted in significantly higher TQ values. In September of both years, the TQ was not significantly different.

Clipping Yield

Clipping yield was significantly affected by the main effect of N level, TE regime and harvest date. Increases in clipping were exhibited as N rate increased. Control plots

receiving 0 kg N ha^{-1} exhibited 2.83 g m^{-2}, whereas 3.42, 3.99, and 4.72 g m^{-2} clipping yields were displayed for 4.9, 9.8 and 14.7 kg N ha^{-1} respectively. TE decreased clipping yield significantly with control plots exhibiting 4.29 g m^{-2}, a 32% increase over plots receiving TE at 3.19 g m^{-2}. The date in which clippings were harvested significantly affected the yield. The greatest yield occurred in August 2009 at 5.97 g m^{-2}, followed by 4.76, 2.69, and 1.53 g m^{-2} for July 2010, August 2010, and September 2009 respectively. N level* harvest date interaction took place that exhibited that in 2009, both harvests resulted in similar clipping yields across all N levels. This is probably due the duration of treatments in 2009. In 2010, a clear and significant N level affect was exhibited at both harvest dates. At each harvest in 2010, increasing N rates led to increased clipping yields. In July 2010, applications of 14.7 kg N ha^{-1} resulted in a clipping yield of 6.94 g m^{-2}, whereas N levels of 9.8, 4.9, and 0 kg N ha^{-1} displayed yields of 5.38, 3.99, and 2.74 g m^{-2} respectively. The August harvest exhibited the same trend with clipping yields of 4.30, 3.19, 2.19, and 1.07 g m^{-2} for each N level. Lastly, TE regime* harvest date interaction was significant and displayed that at each harvest, TE reduced clipping yield over the control plots not receiving TE (Table 7.3).

N Concentration

N concentration of leaf tissue, expressed in %DW, was significantly affected by N rate (Table 7.2). As N rate increased, the N concentration of the leaf tissue increased. Under 14.7 kg N ha^{-1} treatments, leaf tissue N concentration reached 3.14 %/DW, whereas control treatments receiving 0 kg N ha^{-1} exhibited 2.20 % N / DW. TE regime had no effect on N concentration of leaf tissue. However, there was a main effect of time

on N concentration. Over the course of the study there were four harvests. In 2009, harvests took place in August and September and in 2010, July and August. The August 2009 harvest exhibited the greatest overall N concentration in leaf tissue at 3.40%/DW. Each harvest was significantly different with both 2009 harvests exhibited higher N contents than both 2010 harvests. The lowest N concentration in leaf tissue was exhibited in August 2010 at 2.22 %/DW. An N rate*harvest date interaction took place. During the August 2009 harvest, each fertility level resulted in significantly higher N concentration in the leaf tissue, except 4.9 kg ha^{-1} which was similar to the control fertility level. The September 2009 harvest resulted in N concentrations being similar in all treatments except for plots receiving 9.8 kg ha^{-1} N, which were significantly higher than control treatments (0 kg N ha^{-1}). The July 2010 harvest exhibited significant differences for each fertility level. N concentrations were highest in 14.7 kg N ha^{-1} treatments (3.46% DW) and decreased as fertility level decreased (1.60 % DW, Control). The August 2010 harvest exhibited the same trend as the previous month, with N concentrations in leaf tissue decreasing as the fertility level decreased. (2.81 % DW – 14.7 kg ha, 1.64% DW-Control).

Phosphorus Concentration

P concentration of leaf tissue expressed in %DW was significantly affected by N rate. 9.8 and 14.7 kg N ha^{-1} rates exhibited similar P concentrations (0.42 and 0.44 %/DW) and were both significantly higher than 4.9 and 0 kg N ha^{-1} treatments (0.39 and 0.37 %DW). TE regime had no affect on P concentrations throughout the study. Harvest date exhibited a significant change in P concentration. August 2009 and July

2010 harvests were similar at (0.45 and 0.43 %DW) and significantly higher than September 2009 and August 2010 harvests, which were both significantly different at 0.39 and 0.36 % DW respectively. An N rate*harvest date interaction took place. Over the course of the study there were four harvests. In 2009, harvests took place in August and September and in 2010, July and August. During August and September 2009 harvests there weren't any significant differences among N fertility levels. The July 2010 harvest displayed that 14.7 and 9.8 kg N ha^{-1} treatments resulted in similar P concentration in leaf tissue. 4.9 kg N ha^{-1} and control treatments (0 kg N ha^{-1}) exhibited similar P concentration in leaf tissue, and were significantly less than 14.7 and 9.8 kg N ha^{-1} treatments. August 2010 harvest exhibited a similar trend as the July 2010 harvest. 14.7 and 9.8 kg N ha^{-1} treatments resulted in the same P concentration in leaf tissue. Secondly, 9.8 and 4.9 kg N ha^{-1} treatments were similar, however 4.9 was exhibited significantly less P concentration than 14.7 kg N ha^{-1} treatments. Control treatments receiving 0 kg N ha^{-1} had the lowest P concentration in leaf tissue.

Potassium Concentration

Potassium concentration of leaf tissue was not significantly affected by N rate. However TE regime influenced K^+ concentrations in leaf tissue. The main effect of TE reduced K^+ concentrations in leaf tissue from 1.14 %DW under control to 1.05 %DW under .017 L a.i ha^{-1} TE. Harvest date significantly influenced K^+ concentrations of leaf tissue. Each harvest was significantly different from each other with July 2010 averaging the greatest K^+ concentration at 1.34 %DW and decreasing to 1.12, 1.05 and 0.87 % DW for August 2010, August 2009, and September 2009 respectively. An N rate*harvest date

interaction took place. Over the course of the study there were four harvests. In 2009, harvests took place in August and September and in 2010, July and August. The August 2009 harvest didn't exhibit any differences in K^+ concentration among N fertility levels. For the September 2009 harvest, the control, 4.9 and 9.8 kg N ha^{-1} treatments were not significantly difference in K^+ concentration, however 14.7 kg N ha^{-1} treatments were significantly decreased. The July 2010 harvest exhibited differences in K^+ concentration due to N fertility levels. 14.7 kg N ha^{-1} treatments were significantly higher than 4.9 and control treatments. However, 14.7 and 9.8 kg N ha^{-1} were not significantly different in K^+ concentration in leaf tissue. Similar results were displayed for the August 2010 harvest. 14.7 and 9.8 kg N ha^{-1} treatments exhibited similar in K^+ concentration. However, they were both significantly higher in K^+ concentration than 4.9 and control treatments, which were significantly different from each other at 1.08 and 0.95 % DW respectively. A TE regime*harvest date interaction took place. No significant differences in K^+ concentration among TE regimes for the August 2009 September 2009 harvests. For the July and August 2010 harvests, control treatments of TE (0 L ai. ha^{-1}) resulted in significantly higher K^+ concentrations in leaf tissue. In July 2010, an 8 % decrease in K^+ concentration was exhibited due to the addition of TE. (1.39, 1.28 % DW). In the following month, a 14.88 % decrease in K^+ concentration was seen due to applications of TE.

Ball Roll Distance

Ball roll distance (BRD) was significantly affected by N rate. The greatest BRD was displayed by the control treatments receiving 0 kg/ha N and continued to decrease as

N rate increased. BRD of 245.13, 235.93, 231.19, and 229.80 cm were exhibited as N rate increased (Figure 7.3). A 10 cm decrease of BRD was displayed as N rate increased from 0 to 14.7 kg ha^{-1}weekly. McCarty (2011) found that for each kilogram of N applied annually, per hectare 10 cm decreases in speeds occur. The main effect of TE treatments did not influence BRD. The month in which readings were taken significantly affected BRD. Overall BRD increased as turf growth slowed late into the season. BRD values of 214.95 cm were exhibited in August and increased to 245.45 cm by November, an increase of 12.42%. The main effect of year was significant with 2010 BRD values being significantly higher than 2009 at 240.44 and 230.58 cm respectively. Significant N rate*TE regime interactions were seen. Control (0 kg N ha^{-1}) treatments benefited from TE applications, increasing BRD by 6.77 cm. However 14.7 kg N ha^{-1} treatments displayed decreased BRD when TE was applied. BRD values for 4.9 and 9.8 kg N ha^{-1} treatments did not differ with TE regime. A significant N level*month interaction occurred. For all months, control plots exhibited the highest BRD values (Figure 7.2). In August and September, BRD increased as N level decreased, with each N level being significantly different. However, in October 4.9, 9.8 and 14.7 kg N ha^{-1} N levels resulted in similar BRD values. During November the same trend was exhibited. A TE regime* month interaction exhibited varied results. During the months of August and September TE increased BRD over plots not receiving TE, however the opposite was true for October and November where non TE plots exhibited significantly higher BRD values. Like TQ, an N level*year interaction was exhibited, however during 2009 no difference in BRD was seen across N levels. In 2010, the BRD increased as N level decreased.

Control treatments in 2010 resulted in the highest BRD values, at 260.84 cm, whereas 9.8 and 14.7 kg N ha^{-1} treatments resulted in BRD values of 230 cm. TE applications in 2009 resulted in significantly higher BRD values than non TE plots, however in 2010 there was not any significant difference. A month*year interaction was exhibited. During 2009, the highest BRD values were displayed in October and November and were not significantly different. Overall values in September averaged 234.32 cm and BRD values in August were the lowest of all at 204.32 cm. For 2010, November BRD values were significantly higher than all other months at 248. 97 cm. September and October exhibited similar BRD values in 2010, and August displayed the lowest BRD in 2010 at 225.60 cm.

Trufirm

Firmness was significantly affected by the main effects N level, TE regime, month and year. An increase in N rate decreased surface firmness, presumably due to the increase in leaf tissue leading to a more cushioned response. 14.7 kg N ha^{-1} weekly resulted in a Trufirm reading of 1.26 cm (depth of penetration). As N rate decreased, surface firmness increased, resulting in readings of 1.22, 1.19, and 1.16 cm penetration for 9.8, 4.9 and 0 kg N ha^{-1} (Figure 7.4). Surface firmness as measured by the Trufirm was influenced by TE regime. A slight, but significant 0.015 cm decrease in penetration was exhibited under TE. Month had a significant effect on surface firmness. Penetration varied from 1.23 to 1.19 cm in the months recorded. October exhibited the most firmness at 1.19 cm of penetration, similar to November at 1.20 cm, which were both more firm than August at 1.23 cm. The plots in 2010 were more firm than 2009. A decrease in

overall penetration from 1.24 to 1.18 cm was exhibited. A significant N level*TE regime interaction was exhibited. Under 4.9 kg N ha^{-1} treatments, applications of TE resulted in an increase in surface firmness (1.22 cm to 1.16 cm). Whereas at 14.7 kg N ha^{-1}, applications of TE decreased firmness from 1.24 to 1.28 cm. An N level *year interaction was displayed. 4.9 kg N ha^{-1} and control plots exhibited firmer conditions in 2010. In 2009, 14.7 kg N ha^{-1} exhibited the greatest penetration at 1.26 cm, all other N levels resulted in similar firmnesses. In 2010 there was more separation between N levels and their respective firmness levels. 14.7 kg N ha^{-1} displayed the greatest penetration at 1.26 cm which was consistent with 2009. Readings of 1.21, 1.15, and 1.10 cm were displayed for 9.8. 4.9 and control N levels respectively. Month*year interaction was exhibited. October was the only month where firmness levels were similar in both years. All other months displayed firmer conditions in 2010.

Thatch Depth

Thatch depth was significantly influenced by the main effect of N level. 4.9 kg N ha^{-1} and control treatments resulted in similar overall thatch depths of 2.30 and 2.24 cm respectively. 14.7 and 9.8 kg N ha-1 treatments were similar at 2.14 and 2.11 cm. TE applications had no affect on thatch depth. There was a significant effect of month and year on thatch depth. November exhibited the greatest overall thatch depth at 2.41 cm. October displayed the second greatest thatch depth at 2.24 cm, while September and August had similar depths at 2.11 and 2.03 cm respectively. Overall, thatch depths in 2010 were greater than 2009 at 2.29 and 2.11 cm respectively. An N level* year interaction was significant. During 2010, all N levels resulted in similar thatch depths.

However, in 2009 14.7 and 9.8 kg N ha^{-1} exhibited significantly less thatch depth than control or 4.9 kg N ha^{-1} treatments. Month*year was also significant. In 2009, October and November exhibited similar thatch depths at 2.32 and 2.23 cm respectively. August exhibited an overall thatch depth of 2.09 cm while September had the lowest at 1.80 cm. In 2010, the same trend was not exhibited. November displayed the greatest thatch depth at 2.59 cm, significantly higher than September at 2.42 cm, October (2.16 cm) and August 1.98 cm.

Thatch Accumulation (Loss on Ignition)

LOI was significantly influenced by the main effect of year. In 2010, LOI values were significantly higher than 2009 at 1259.7 and 841.54 g m^{-2} respectively.

Discussion

The total amount of N supplied was different in the two years that this study was conducted. In 2009 total N treatments lasted 7 weeks totaling – 34.3, 68.6, and 102.9 kg N ha^{-1}, respectively, for the three treatments however, in 2010 total N treatments lasted 15 weeks resulting in 73.5,147, and 220.5 kg N ha^{-1}, respectively, for the three treatments. Due to this difference in total fertility, the results from 2010 were more clearly defined and separated between N levels. An N level*year interaction was significant for every parameter except LOI (Table 7.1). N level significantly influenced N and P concentrations in leaf tissue over the course of the study. McCarty (2011) stated that zoysiagrasses should generally not be fertilized with more than 146.49 kg^{-1} ha^{-1} over the growing season. Due to this recommendation and desire to reduce overall inputs in turfgrass management, weekly spoon feeding low concentrations of N over the course of

the growing season is suggested. As N level increased during the study, putting green performance as indicated by BRD and surface firmness began to decrease. Therefore fertilization of 147 kg^{-1} N ha^{-1} or less is a good starting point for Diamond zoysiagrass putting green management, with additional quick release N sources following cultivation events to promote growth and recovery.

Although the main effect of N level did not influence K^+ concentration in leaf tissue, TE regime did. Overall reductions were seen under TE application, although the potential benefits of TE applications far outweigh the potential reduction in K^+ concentration. Soper et al. (1988) found that thatch and tiller density of 'Meyer' zoysiagrass was increased by N applications. No increase in thatch depth was observed under PGR treatments, which agrees with findings of Soper et al. (1988). Additional research in utilizing TE in Diamond putting green performance needs to be conducted.

PGR applications can potentially increase ball roll distance due to the reduction in vertical growth and leaf surface area; however the main effect of TE did not influence overall BRD in this study (Table 7.1). There are many cultivation and management techniques utilized to improve putting green performance including rolling, topdressing, decreasing HOC, and water management. Salaiz et al. (1995) documented increases in BRD with a reduction of HOC, however a decrease in TQ and root production was exhibited. A decrease in HOC, increase in PGR application rate, rolling, and cultivation could increase ball roll on Diamond putting greens. A conservative 3.2 mm HOC was utilized in the study however; Stiglbauer et al. (2009) found that Diamond can be maintained at a 2.5 mm HOC without scalping or winter survival issues. Increases in

BRD due to reductions in have been well documented in bentgrass and seashore paspalum (Fagerness et al., 2000; Kopec et al., 2007; Pease et al., 2011). In addition to increases in BRD, annual bluegrass putting greens can be maintained by increasing mowing and/or rolling without increasing anthracnose severity (Inguagiato et al., 2009).

No cultivation was performed during the 2 year study period. Thatch and mat management techniques need to be evaluated in Diamond zoysiagrass putting greens. Extensive cultivation as tested by Hollingsworth et al. (2005) on ultradwarf bermudagrass would not be advised due to the slow growth habit of Diamond zoysiagrass. Hollingworth et al. (2005) found that extensive cultivation on ultradwarf bermudagrasses was too intensive to maintain acceptable TQ and playability. Although a slow growth habit is desirable in many turf management scenarios, it could hinder the playability of Diamond zoysiagrass putting surfaces for a prolonged time following core aeration. McCarty et al. (2007) showed that core aeration decreased BRD up to 14 days after cultivation on creeping bentgrass putting green surfaces. This reduction in BRD could be much longer due to the slow growth habit of Diamond. Hollingsworth et al. (2005) found that thatch depth was not affected by N source. However, in this study, N level did influence total thatch depth. More interesting was the main effect of time and how it showed thatch accumulation throughout the growing season (Figure 7.5). Moisture readings were not taken during this study. Surface firmness could be influenced on the moisture in the upper 2.54 cm of the soil/thatch. There was a significant month and year main effect. To more fully understand what might cause these fluctuations in firmness additional moisture data must be taken. Diamond zoysiagrass has the potential to be

installed and utilized successfully in putting green scenarios. A balance between TQ and putting green performance needs to be found to determine optimum N fertility level. Additional research needs to be conducted to examine N fertility management, lowered HOC, increased PGR rate/application intervals, rolling, and other cultivation techniques for thatch and mat control.

Conclusions

Diamond zoysiagrass has the ability to become another warm-season turfgrass option for putting greens in the southern transition zone. However, before widespread use of fine leaf zoysiagrass cultivars is considered many issues need to be resolved. Based on finding of this project, N fertilization of Diamond zoysiagrass in putting green applications should begin with 147 kg^{-1} N ha^{-1} or less over the growing season. Additional quick release N sources should be used following cultivation events to promote growth and recovery. As total N input surpassed 147 kg^{-1} N ha^{-1} putting green performance suffered. An obvious increase in thatch depth and accumulation was displayed during the two year study. Cultivation, surface management, PGR use, and fertility regimes need to be determined to optimize putting green performance and overall turfgrass health of Diamond zoysiagrass in putting green scenarios.

Table 7.1. Surface firmness (cm of penetration) of Diamond zoysiagrass as influenced by N rate, TE level, and year across three rating dates at the Cliffs Environmental Turfgrass Research Facility in Marietta, SC.

Main effects	August	September	October	November
	----------cm of penetration----------			
NRate (N)				
0	1.20	1.15	1.14	1.15
4.9	1.20	1.20	1.17	1.19
9.8	1.23	1.23	1.21	1.21
14.7	1.28	1.28	1.25	1.24
$LSD_{0.05}$	0.04	0.04	0.04	0.03
TE Level (TE)				
Control	1.23	1.23	1.19	1.21
0.017 L a.i ha^{-1}	1.22	1.20	1.19	1.19
Year (Y)				
2009	1.28	1.24	1.20	1.22
2010	1.18	1.19	1.18	1.17
	ANOVA			
Source of variation				
N	**	***	***	***
TE	NS	NS	NS	NS
Y	***	**	NS	**
N*TE	NS	NS	*	*
N*Y	NS	*	*	**
TE*Y	NS	NS	NS	NS
N*TE*Y	NS	NS	NS	NS

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

Table 7.2. Ball roll distance (cm) of Diamond zoysiagrass as influenced by N rate, TE level, and year across three rating dates at the Cliffs Environmental Turfgrass Research Facility in Marietta, SC.

Main effects	August	September	October	November
	----------cm----------			
NRate (N)				
0	222.8	250.9	252.9	254.6
4.9	217.8	244.4	240.8	240.7
9.8	212.0	234.0	237.6	240.9
14.7	207.0	228.9	237.3	245.4
$LSD_{0.05}$	3.99	4.50	4.46	5.37
TE Level (TE)				
Control	212.6	235.7	244.0	248.4
0.017 L a.i ha^{-1}	217.3	243.4	240.3	242.4
Year (Y)				
2009	204.3	234.5	241.7	241.9
2010	225.6	244.6	242.5	248.9
ANOVA				
Source of variation				
N	***	***	***	***
TE	**	***	*	**
Y	***	***	NS	***
N*TE	NS	**	NS	*
N*Y	***	***	***	***
TE*Y	NS	NS	***	NS
N*TE*Y	NS	NS	NS	NS

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

Table 7.3. Turf Quality of Diamond zoysiagrass as influenced by N rate, TE level, and year at the Cliffs Environmental Turfgrass Research Facility in Marietta, SC.

Main effects	August	September	October	November
	----------(1-9, >6)----------			
NRate (N)				
0	6.02	6.06	5.86	5.09
4.9	6.93	7.12	6.95	6.25
9.8	7.15	7.04	7.05	6.62
14.7	7.23	6.95	7.15	6.59
$LSD_{0.05}$	0.35	0.27	0.27	0.27
TE Level (TE)				
Control	6.88	6.81	6.69	6.15
0.017 L a.i ha^{-1}	6.78	6.77	6.82	6.13
Year (Y)				
2009	6.97	6.75	7.0	6.43
2010	6.69	6.84	6.51	5.85
ANOVA				
Source of variation				
N	***	***	***	***
TE	NS	NS	NS	NS
Y	*	NS	***	***
N*TE	NS	NS	NS	NS
N*Y	***	***	***	***
TE*Y	NS	NS	NS	NS
N*TE*Y	NS	NS	NS	NS

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

Table 7.4. Thatch depth of Diamond zoysiagrass as influenced by N rate, TE level, and year at the Cliffs Environmental Turfgrass Research Facility in Marietta, SC.

Main effects	August	September	October	November
	----------cm----------			
NRate (N)				
0	2.11	2.17	2.28	2.42
4.9	2.08	2.20	2.37	2.53
9.8	1.92	2.0	2.15	2.36
14.7	2.02	2.07	2.16	2.32
$LSD_{0.05}$	NS	NS	0.18	NS
TE Level (TE)				
Control	2.02	2.14	2.24	2.39
0.017 L a.i ha^{-1}	2.04	2.08	2.24	2.42
Year (Y)				
2009	2.09	1.80	2.32	2.23
2010	1.98	2.42	2.16	2.59
	ANOVA			
Source of variation				
N	NS	NS	*	NS
TE	NS	NS	NS	NS
Y	NS	***	*	***
N*TE	NS	NS	NS	NS
N*Y	NS	NS	**	NS
TE*Y	NS	NS	NS	NS
N*TE*Y	NS	*	NS	NS

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

Table 7.5. Clipping yield of Diamond zoysiagrass as influenced by N rate and TE level at the Cliffs Environmental Turfgrass Research Facility in Marietta, SC.

Main effects	2009		2010	
	August	September	July	August
	----------g m^{-2}----------			
NRate (N)				
0	5.90	1.28	2.62	1.02
4.9	5.78	1.38	3.81	2.09
9.8	5.49	1.56	5.14	3.05
14.7	5.65	1.63	6.62	4.11
$LSD_{0.05}$	NS	NS	0.78	0.39
TE Level (TE)				
Control	6.44	1.75	4.92	3.27
0.017 L a.i ha^{-1}	4.97	1.17	4.17	1.86
ANOVA				
Source of variation				
N	NS	NS	***	***
TE	***	**	*	***
N*TE	NS	NS	NS	***

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

Table 7.6. N concentration of Diamond zoysiagrass leaf tissue as influenced by N rate and TE level at the Cliffs Environmental Turfgrass Research Facility in Marietta, SC.

Main effects	2009		2010	
	August	September	July	August
	----------g m^{-2}----------			
NRate (N)				
0	3.15	2.44	1.60	1.64
4.9	3.35	2.66	2.16	2.01
9.8	3.53	2.74	2.67	2.42
14.7	3.60	2.69	3.46	2.81
$LSD_{0.05}$	0.12	NS	0.47	0.14
TE Level (TE)				
Control	3.38	2.68	2.42	2.22
0.017 L a.i ha^{-1}	3.43	2.59	2.53	2.22
	ANOVA			
Source of variation				
N	***	NS	***	***
TE	NS	NS	NS	NS
N*TE	*	*	NS	NS

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

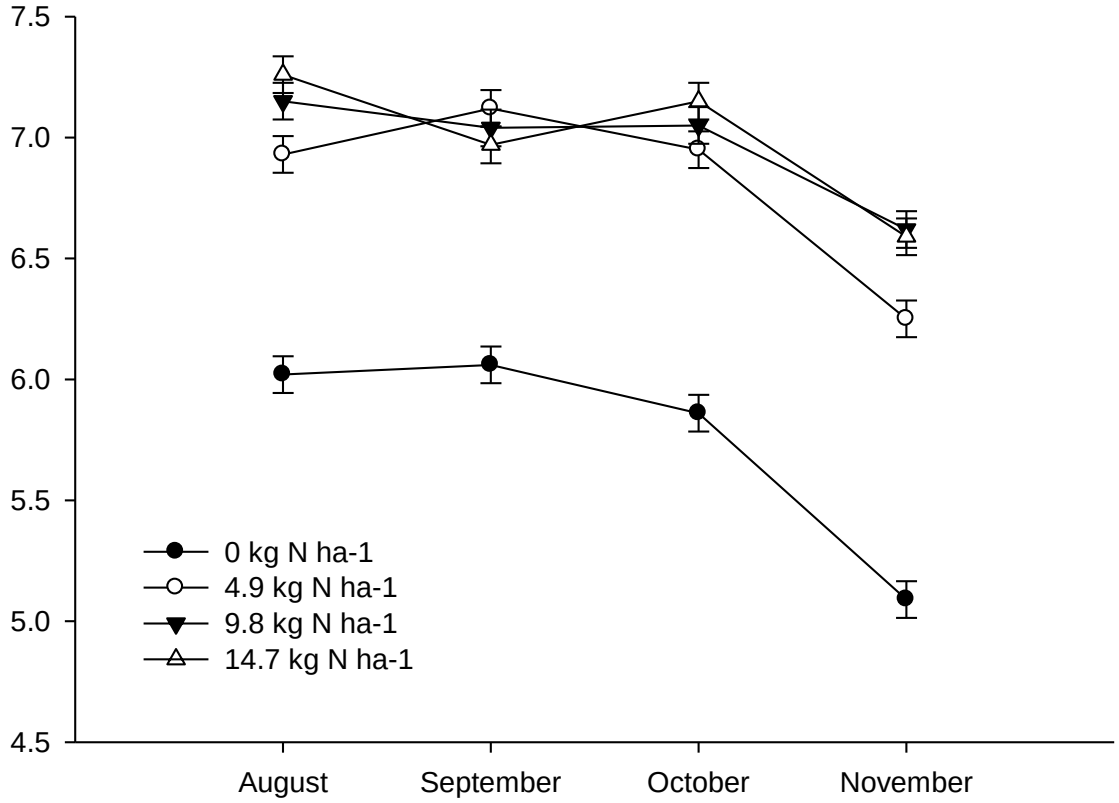


Figure 7.1. Turf quality as affected by N level and time (1-9, >6 acceptable) Means were separated at P≤0.05 by protected LSD.

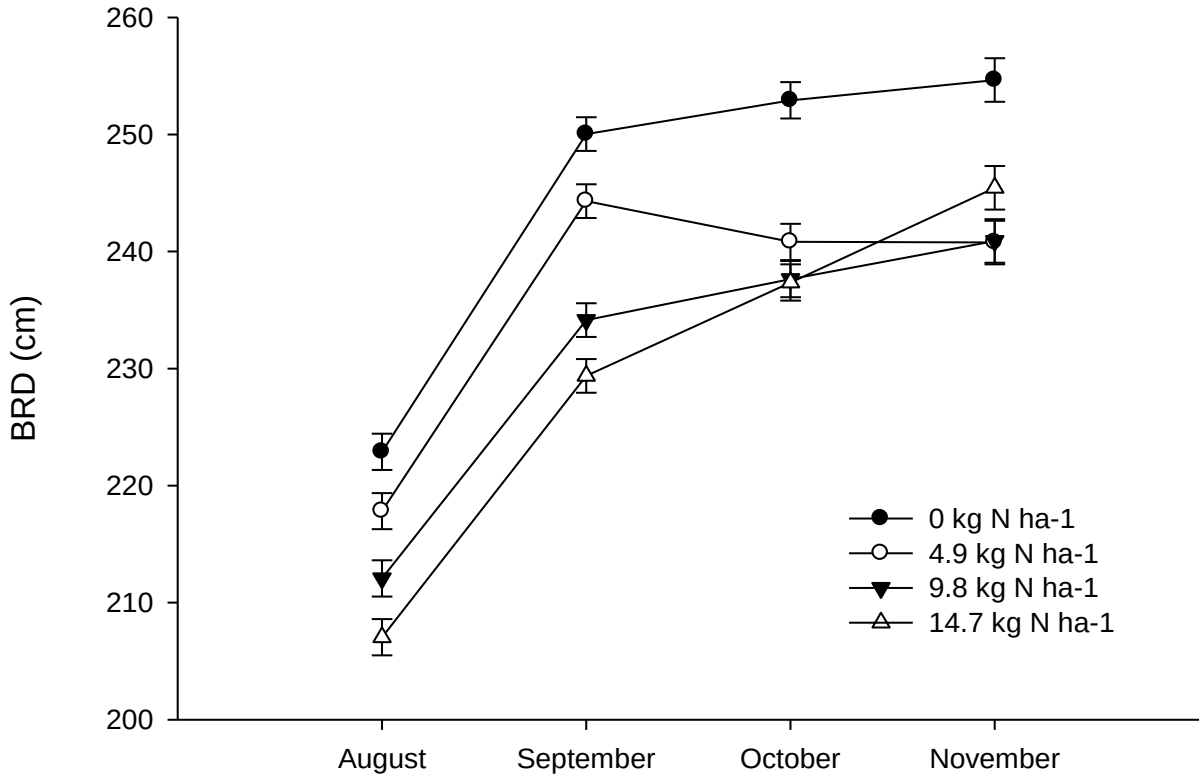


Figure 7.2. Ball roll distance (cm) as affected by N level and time. Means were separated at P≤0.05 by protected LSD.

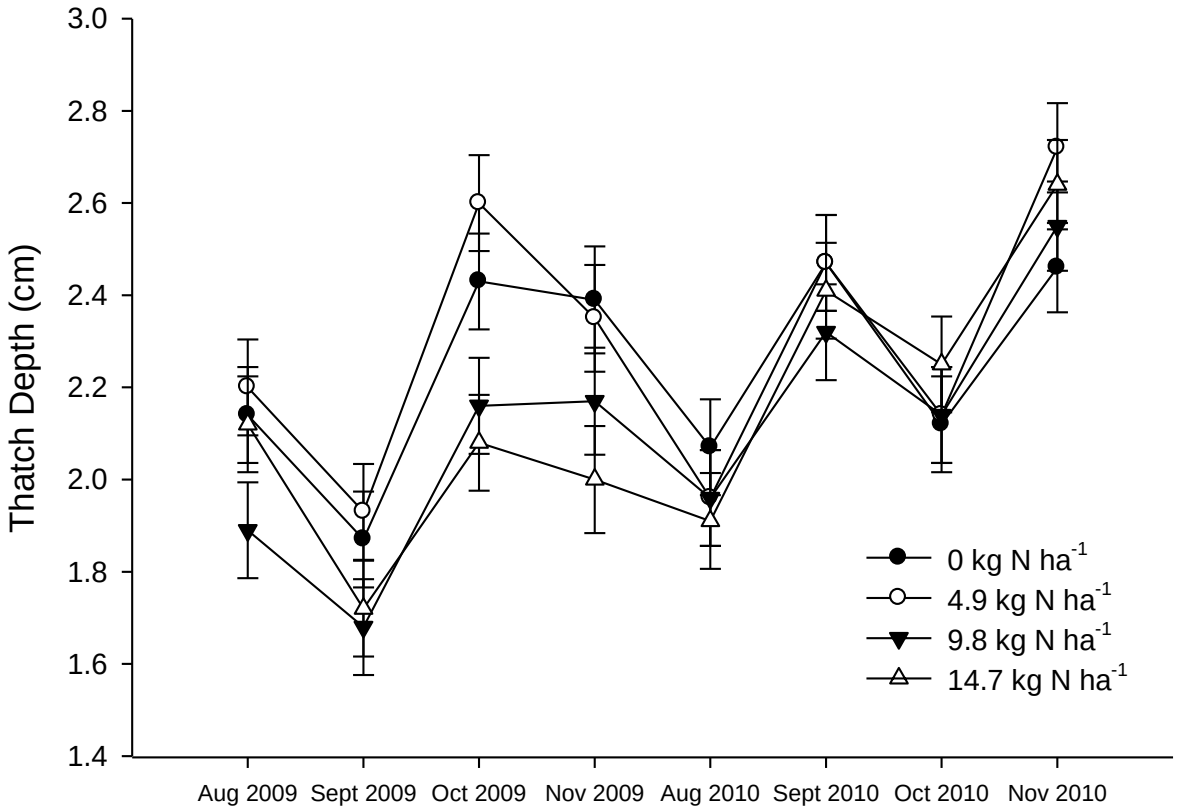


Figure 7.3. Main effect of N level on thatch depth over 4 harvest dates and 2 years. Means were separated at P≤0.05 by protected LSD.

CHAPTER VIII

SUMMARY AND PERSPECTIVES

Primary objectives of this dissertation were to determine the effect of urea N delivery method on warm-season turfgrass under salinity stress, the influence of Ni^{2+} supplementation on urea N metabolism, and the consequence of Ni^{2+} toxicity on urea N metabolism and turfgrass performance. Secondary objectives include determining if Ni^{2+} supplementation aids foliar uptake of urea N. In addition, a field study was conducted to examine Diamond zoysiagrass putting green management through various N fertility rates and applications of plant growth regulators.

A greenhouse study was conducted in 2009 and repeated in 2010 examining foliar and root applied urea N to five warm-season turfgrasses under salinity stress. Foliar and root applied urea N resulted in similar N, P, and K^+ concentrations in the leaf tissue of all genotypes. However, overall leaf tissue Na^+ concentrations at the conclusion of the study were significantly higher in most genotypes excluding Seadwarf, receiving root applied urea N than foliar applied urea. The increase in Na^+ concentration in leaf tissue due to root applied urea was not expected and could be due to complex soil salinity related factors and the N form being absorbed by root tissue. Liu et al. (2003) showed that specific high-affinity transporters and aquaporins are responsible for urea uptake in the root-zone. Due to this fact, urea hydrolysis in the soil and the N form being absorbed by plants could influence the pH in the root zone and stimulate uptake of Na^+ in turfgrasses under salinity stress. Future research should be conducted to examine the level of urease activity in the soil, soil pH, and N form being absorbed following applications of root

applied urea N under salinity stress to determine what is causing the stimulation of Na^+ uptake. In addition, research examining N absorption needs to be fully examined to determine nutrient uptake and assimilation in an effort to maximize N use efficiency in salt-affected areas. Finally, Ni^{2+} nutrition and supplementation of warm-season turfgrasses needs to be researched due to Ni^{2+}'s essential role in the hydrolysis of urea in an effort to decrease N losses following urea N fertilization.

A second study was conducted in the greenhouse further examining urea N foliar fertilization with Ni^{2+} supplementation under moderate salinity stress. Diamond zoysiagrass and TifEagle ultradwarf bermudagrass were selected due to their performance in the first study and subjected to weekly foliar urea N applications, three supplemental Ni^{2+} levels, and moderate salinity stress. Results of this study revealed a stimulation of urease activity and an increase in total amino acids due to Ni^{2+} supplementation. However, a reduction in total N concentration was displayed over the course of the study which can occur when plants grow on a single N source. Due to findings in this study, multiple N sources (NO_3^-, NH_4^+) should be utilized in turfgrass management. Increases in overall growth were displayed under moderate salinity stress in Diamond and TifEagle. This result supports the theory that warm-season turfgrasses require greater concentrations of Na^+ to maintain optimal growth.

Although Ni^{2+} deficiency is not commonly thought of as an issue in turfgrass management; there is evidence that highlights Ni^{2+}'s essential role in plant metabolism and deficiency in pecan. Nickel deficiency in pecan and other fruit trees is becoming more common and it is possible that many horticulture crops possess a "hidden hunger"

for Ni^{2+} (Wood et al., 2004a; 2004b). In addition, Wood et al. (2012) has recorded the positive effects of Ni^{2+} supplementation on disease management of fruit trees. Fe and elevated concentrations of transition metals (V, Cr, Co, Cu^{2+}, Zn^{2+}, and Mo) induced Ni^{2+} deficiency in pecan (Wood, 2011). Due to the popularity of foliar applied Fe and micronutrient fertilizers in turfgrass management to correct nutrient deficiencies and increase turf color/quality, it is possible that Ni^{2+} deficiencies are occurring.

Further research needs to be conducted to examine Ni^{2+} supplementation on warm season turfgrass supplied with various N sources and rates. The significance of Ni^{2+} supply depends on N source and critical concentrations of Ni^{2+} in turfgrass tissue need to be determined in those scenarios. A comprehensive investigation of Ni^{2+} nutrition in turfgrasses also needs to be conducted to determine micronutrient interactions and deficiencies. Lastly, the positive and negative effects of Ni^{2+} supplementation need to be determined, including Ni^{2+} toxicity and disease management of turfgrasses.

A third greenhouse study was conducted to examine the effect of Ni^{2+} toxicity on the health and performance of Diamond zoysiagrass and TifEagle bermudagrass. Stimulation of urea N metabolism was displayed through increases in urease activity and amino acid content in leaf tissue. However, increased Ni^{2+} concentration in leaf tissue led to symptoms of toxicity, changes in nutrient status, reduction in growth, decreases in TQ, and leaf necrosis. Findings from this study suggest the critical Ni^{2+} toxicity level in Diamond zoysiagrass and TifEagle bermudagrass begins at a range >25 mg kg^{-1}. Leaf tissue Ni^{2+} concentrations greater than 25 mg kg^{-1} caused reductions in turf growth and symptoms of toxicity.

Additional research needs to be conducted to examine if Ni^{2+} supplementation aids in foliar recovery of applied urea N and if foliar applications of Ni^{2+} can be made. Lastly, research should focus on Ni^{2+} requirement of turfgrasses along with long term ecological, physiological, and environmental implications of Ni^{2+} supplementation in turfgrass environments.

A fourth experiment was conducted in the greenhouse to determine the differences in ^{15}N recovery after foliar and root applications of urea N in three warm-season turfgrasses. Results from this study indicate that foliar and root applications of ^{15}N resulted in overall recovery of 37 and 47% respectively. Significant fertilizer loss (up to 63% of applied N) was exhibited which could be due to a number of reasons including leaching and ammonia volatilization from the leaf surface following applications of urea. Numerous nutrient management strategies have been employed to improve foliar fertility uptake and retention in turfgrass ecology including fertility rate, application timing, N source, and spray volume. Comprehensive research needs to be conducted to account for the significant losses in N following foliar applications of nutrients.

A chapter of this dissertation also addressed the lack of literature on zoysiagrass putting green management. Fine leafed zoysiagrasses have become another warm-season turfgrass option for putting greens, however there are many questions regarding their fertility management and playability in such scenarios. A field study was conducted to examine the effect of foliar N application rates, and plant growth regulator use on Diamond zoysiagrass putting green performance. Based on our results, Diamond

zoysiagrass has the ability to become another warm-season turfgrass option for putting greens in the southern transition zone. However, before widespread use of fine leaf zoysiagrass cultivars is considered many issues need to be resolved. N fertilization of Diamond zoysiagrass in putting green applications should begin with 147 kg^{-1} N ha^{-1} or less over the growing season. Additional quick release N sources should be used following cultivation events to promote growth and recovery. As total N input surpassed 147 kg^{-1} N ha^{-1} putting green performance suffered. Cultivation, surface management, PGR use, and fertility regimes need to be determined to optimize putting green performance and overall turfgrass health of Diamond zoysiagrass in putting green scenarios.

Urea is the most commonly used foliar N source in turfgrass management and agriculture. Although a great N source, nitrogen use efficiency in turfgrass management and crop production is generally below 50% resulting in economical losses and also creating ecological problems like groundwater contamination. Previous research efforts aiming to improve foliar applied N use efficiency in turfgrass management have focused on application technique, without examining the N metabolism and plant physiology.

Before urea can be utilized as an N source by the plant it must be hydrolyzed by the Ni^{2+} dependent enzyme urease in the cytosol. Numerous studies have proven the benefit of Ni^{2+} supplementation in stimulating urea N metabolism and overall plant health in numerous crops. However, there is a lack of research examining Ni^{2+} nutrition and the stimulation of urea N metabolism by Ni^{2+} supplementation in turfgrasses and most horticultural crops. This dissertation began to address the lack of literature on Ni^{2+}

nutrition, supplementation, and toxicity in turfgrass management. Many positive responses were recorded over the course of the experiments. Increases in urease activity within the cytosol led to elevated amino acid pools. However, reductions in total N concentration of leaf tissue were displayed. More research focusing on Ni^{2+} supplementation is necessary to determine the long term effect and potential benefit in N use efficiency.

Many questions remain regarding the effect of Ni^{2+} supplementation and long term ecological issues. Future research regarding urea N metabolism and Ni^{2+} supplementation should focus on foliar applications of Ni^{2+}, the effects of tank mixing Ni^{2+} with foliar urea N fertilizers, Ni^{2+}'s effect on overall plant health and disease management.

APPENDICES

Appendix A

Illustrations

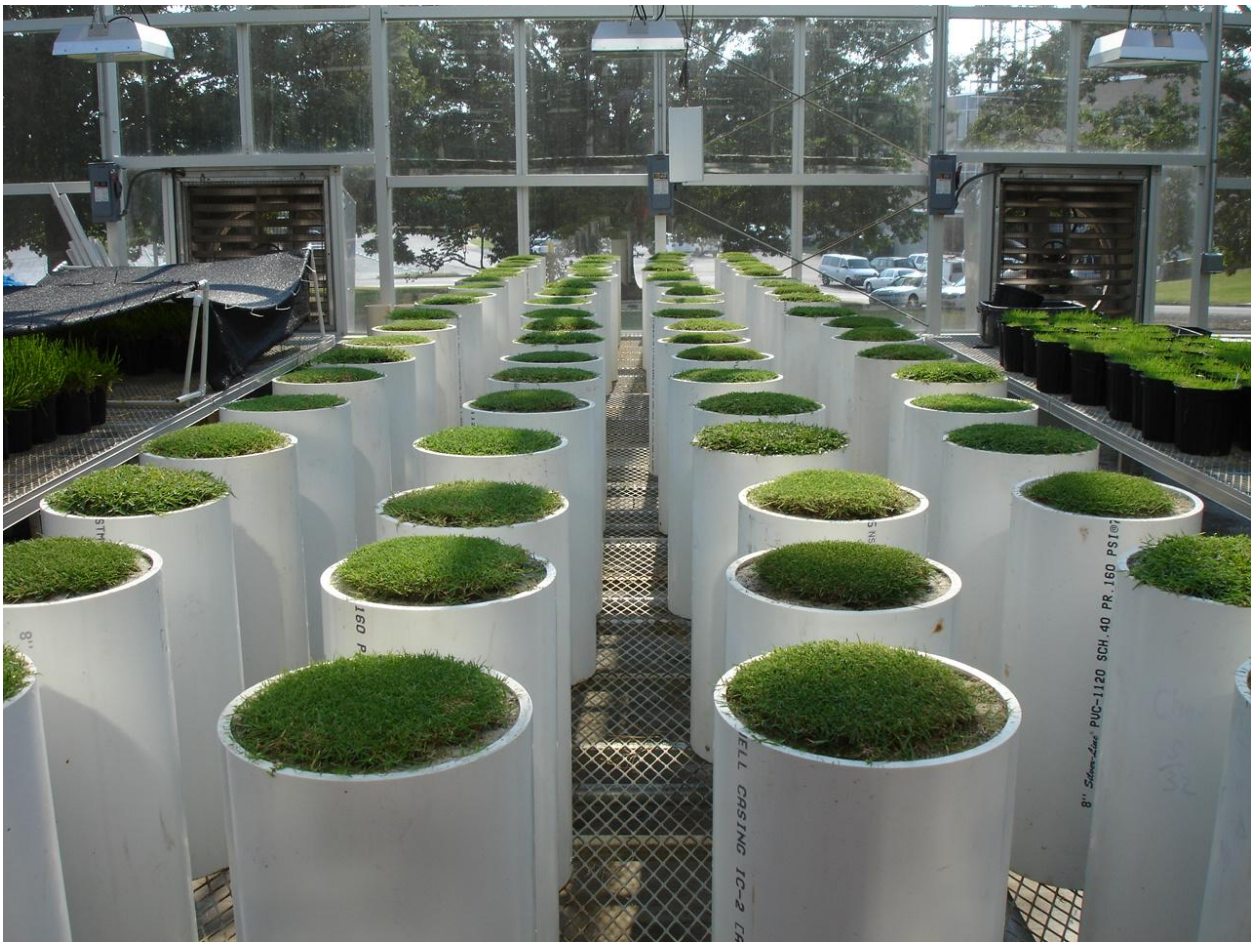


Illustration A-1: Polyvinylchloride lysimeters in the Clemson University Greenhouse Research Facility.

Illustration A-2: Root applications of soluble urea N were made using a 60 ml syringe in the Clemson University Greenhouse Research Facility.

Illustration A-3: Making a root application of soluble urea N in the Clemson University Greenhouse Research Facility.

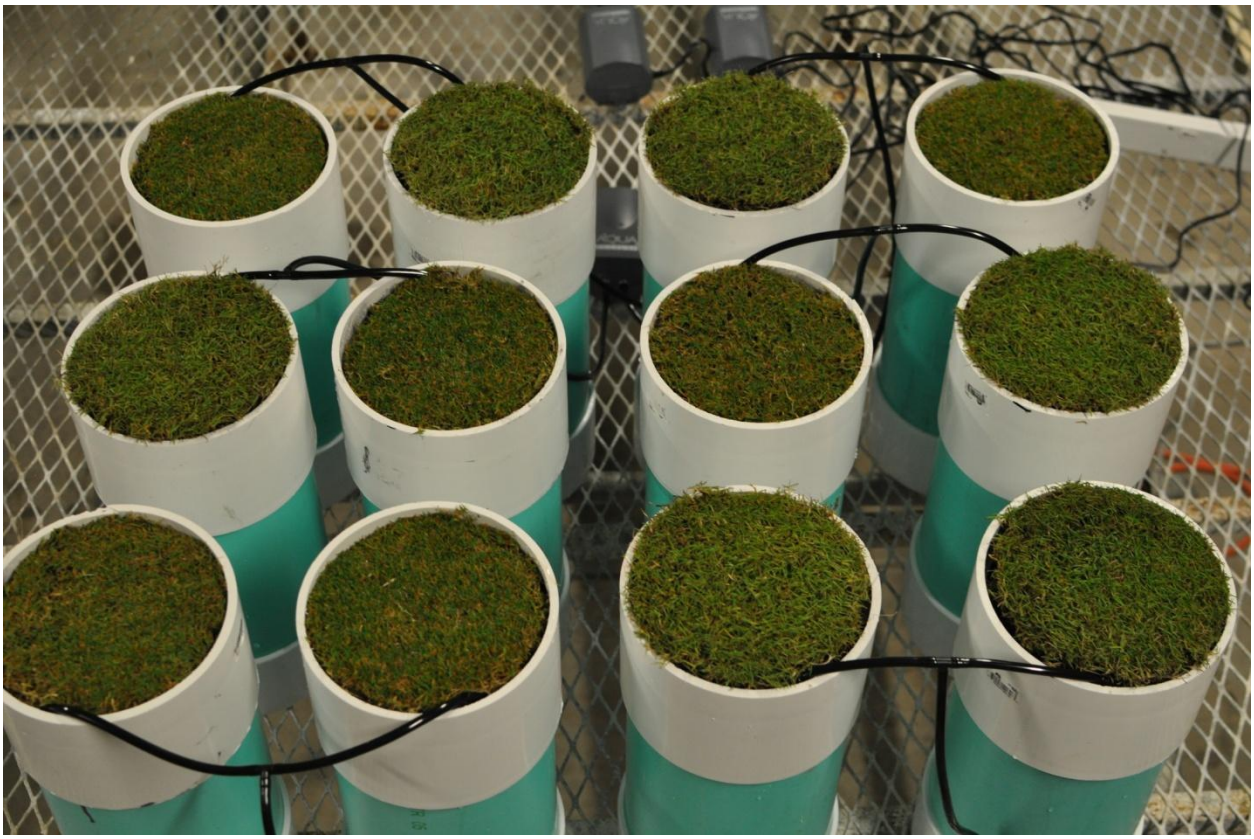


Illustration A-4: Hydroponic pre-culture and establishment in the Clemson University Greenhouse Research Facility.

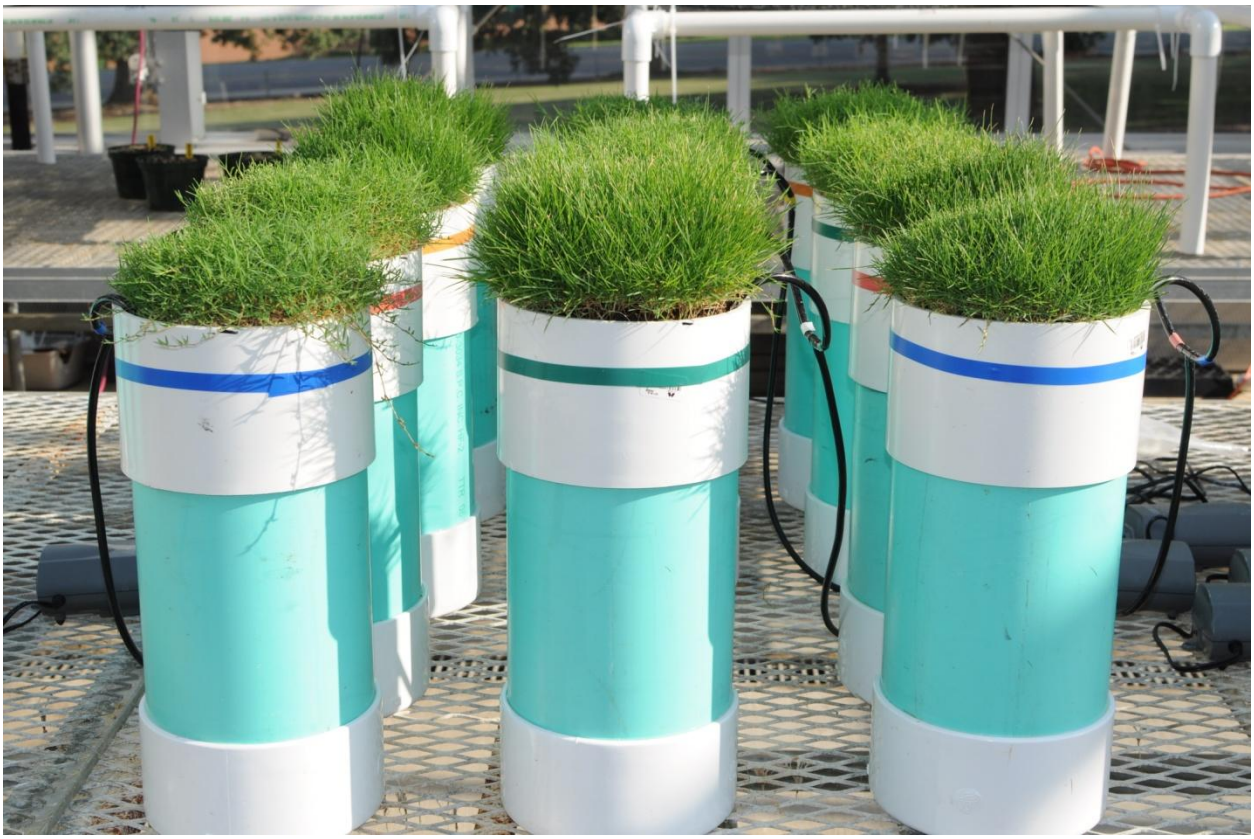


Illustration A-5: Hydroponic culture in the Clemson University Greenhouse Research Facility.

Illustration A-6: Constant aeration was supplied during hydroponic culture in the Clemson University Greenhouse Research Facility.

Illustration A-7: Measuring ball roll distance on Diamond zoysiagrass with a modified Stimpmeter at the Cliffs Environmental Turfgrass Research Facility in Marietta, SC.

Illustration A-8: Measuring surface firmness of Diamond zoysiagrass with Trufirm™ (USGA) at the Cliffs Environmental Turfgrass Research Facility in Marietta, SC.

Illustration A-9: Overview of Diamond zoysiagrass plot located at the Cliffs Environmental Turfgrass Research Facility in Marietta, SC.

Appendix B

Laboratory Procedures

Urease Assay

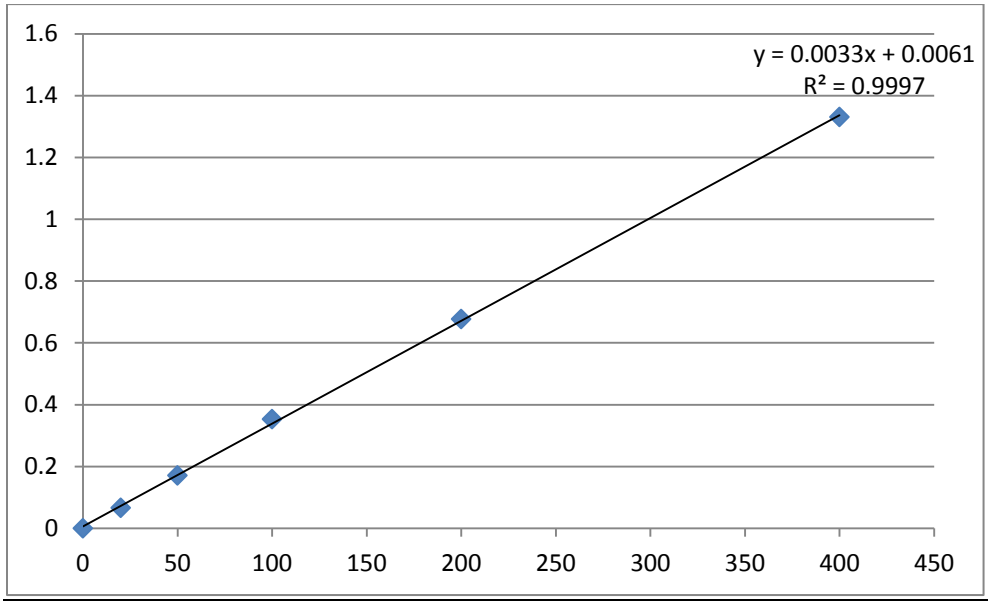
Urease activity was determined spectrophotometrically at 636 nm by the methods of Witte and Medina-Escobar (2001) with modifications. Fresh turfgrass leaf tissue (0.1 g) was extracted in 50 mM phosphate buffer (pH 7.5) containing 1.5% PVPP, 50 mM NaCl, and 1 mMEDTA with mortar and pestle on ice. Immediately before extraction DTT and PMSF were added to a final concentration of 20 and 0.1mM respectively. Following extraction, samples were centrifuged at 4°C for 10 min (14,000 rpm). The supernatants were transferred into new 1.5ml tubes and centrifuged again under the same conditions for 20 min. The clarified extract was removed and kept on ice. Five hundred microliters of Sephadex (G-25 medium) containing 25 mM phosphate buffer, 25 mM NaCl and 0.5 mM EDTA slurry was added to a spin column and pre-spun at 700 g for 1 min. One hundred microliters of each sample was added to the center of the gel and the column was spun again for 2 min at 700 g. Approximately 100 µl was recovered. In 1.5 ml tubes, 90 µl of sample from spin column was added to 1 µl of 5 M urea. The tubes were vortexed and placed into a 50°C heat block for 3 min. Tubes were removed and spun to collect any condensate. Twenty microliter samples were added to 980 µl H_2O, 100 µl phenol nitroprusside and 200 µl hypochloride reagent. After the reagents were added the tubes were closed immediately to avoid ammonia losses, mixed well and placed into a 50°C heat block for a minimum of 20 min after which desired color

development was achieved. Urease activity was based on a standard curve of ammonium chloride. Measurements were made at 636 nm on a spectrophotometer.

Urease reagent preparation

Seven grams of phenol and 34 g nitroprusside (disodium pentacyanonitrosylferrate) were dissolved in 80 ml dH_2O and then made up to 100 ml. This reagent was stored at 4°C in a dark bottle. The hydrochloride reagent was prepared by dissolving 2.96 g NaOH in 140 ml of dH_20, adding 29.74 g Na_2HPO_4*12 H_2O, and dissolving it completely. NaOCl solution (12%) was added and the pH was adjusted to 12.0 with NaOH and the volume was adjusted to 200 ml with dH_2O. This was stored in a dark bottle at room temperature.

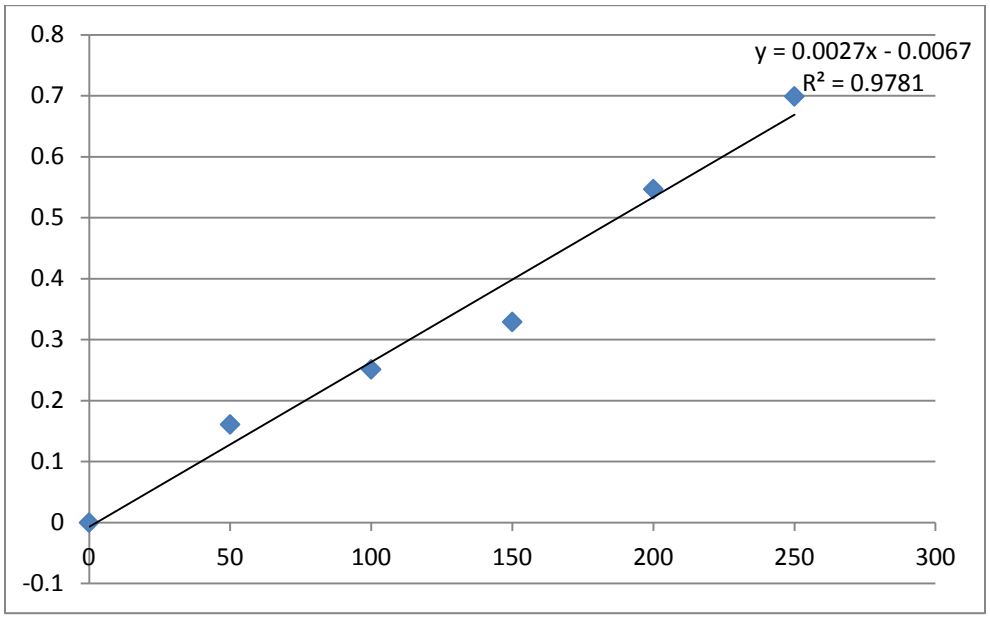
Ammonium Chloride Standard Curve.



Amino Acid Assay

Amino acid content was extracted from fresh leaf tissue and determined spectrophotometrically at 570 nm by the methods of Zhang et al. (2011). One hundred milligrams of fresh turfgrass tissue were ground with a mortar and pestle and extracted in 2.5 ml 0.05 sodium phosphate buffer containing 0.2 mM eDTA and 1 % PVP. Extracts were centrifuged at 13,000 rpm for 20 minutes. The supernatants were collected for the amino acid assay. To each test tube 200 µl extract or standard (glycine solution) were added along with 550 µl dH_2O, and 500 µl diluted cyanide solution (2 ml mM NaCN was brought up to 100 ml final volume with 0.2 M acetate buffer and 500 µl ninhydrin solution [3% {w/v} ninhydrin in ethylene glycol momomethyl ether {2-methoxythanaol}]). The top of each tube was covered with a marble and incubated in boiling water for 20 min. Then 10 ml isopropyl alcohol and water (1:1 v/v) were added to each tube. After vortexing, the tubes were allowed to cool. Absorbance was measured on a spectrophotometer at 570 nm. Total amino acid content was calculated based on a standard curve of glycine.

Glycine Standard Curve.



Appendix C

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Figure 2.3 Reprinted from Plant Science, Volume 180, Claus-Peter Witte, Urea metabolism in plants, 431-477, 2011, with permission from Elsevier.

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